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THE UNIVERSITY OF ALBERTA

FINE STRUCTURE IN FRUITING BODIES OF COPRINUS,

WITH SPECIAL EMPHASIS ON CHROMOSOME STRUCTURE

By

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The undersigned certify that they have read and
recommend to the Faculty of Graduate Studies for acceptance,
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WITH SPECIAL EMPHASIS ON CHROMOSOME STRUCTURE", submitted by
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## ABSTRACT

Electron microscopy was used to reveal the fine structural organization of cellular organelles, especially the nuclei and chromosomes of fruiting bodies of <u>Coprinus</u>. Light microscopy was used to demonstrate the meiotic process, the stages of which were used as indicators of the stages of fruit-body development.

A Golgi apparatus analogous to that described in plant and animal cells has been demonstrated. In thin sections, it has a central area from which many cisternae radiate. From the ends of the cisternae, successions of vesicles appear to be produced. At metaphase I, the Golgi complex becomes extremely proliferated. Vesicles so produced may be the precursors of endomembrane systems. It is postulated that a function of this organelle in <u>Coprinus</u> is as the membrane-system generator.

All membrane systems in <u>Coprinus</u> may probably be homologous. The endoplasmic reticulum may be considered as smooth surfaced, highly branched membrane system which may be continuous with other membranes to form a complex system for intracellular communication. It is suggested that the endoplasmic reticulum may expand to form vacuoles when cells grow old.

Ribosomes and mitochondria are abundant in the basidium of <u>Coprinus</u>. Ribosomes are seen to be strung in chains, the polyribosomes. Mitochondria may be ovoid, oblong, or elongate and may be branched. There are mitochondrial particles similar in size and shape to ribosomes.

Lomasomes have been observed in the basidia of advanced stages of development. They appear to be related to the ageing of the cell.

The basidia have the central role in the development of fruiting bodies of Coprinus. They are the cells of the highest cellular activity having the highest concentration of subcellular particles. Other cell types differ in cellular activity with respect to their relation with the basidia. It is probable that subcellular particles and food supplies are channelled to the basidia during the course of development. As spores are being formed, subcellular particles are transferred from the basidium to the basidiospores.

Meiosis of Coprinus was demonstrated jointly by light microscopy and by electron microscopy. The tripartite synaptinemal complex, characteristic of the synaptic chromosomes, is formed when synapsis begins immediately after nuclear fusion. It comprises two homologous-chromosome axes and a synaptic center. The synaptinemal complex becomes more elaborate with respect to the axial differentiation, when the chromosomes condense. At diplotene, two homologues are far apart, and the synaptic center is no longer present. It is suggested that chromosome condensation during meiosis is the result of packing of chromatin fibrils. The process of chromosome synapsis is envisaged as the process of fibrillar foldings in the formation of the synaptinemal complex.

The centriole is believed to be associated with the outer nuclear membrane. It is made up of microtubules around the ovoid surface, it divides at pachytene, it gives off astral rays at metaphase and is associated with the spindle zone.

The nuclear membrane of <u>Coprinus</u> is porous or annulate. The nuclear annulus is made up of microtubules around its permeter. Inside the pore proper there appear to be two compartments connected to the surrounding microtubules by nine microfibrils, suggesting a 9-2 arrangement.

Interphase chromosomes of cystidia of <u>Coprinus</u> are resolvable by electron microscopy. Each chromosome appears to consist of two 100-Å fibrils which fold laterally to form a chromosome.

The structure of the synaptinemal complex has been studied in some detail. The chromosome axes and the lateral loops alike are composed of 100 Å fibrils. The axes have greater aggregates of fibrils than the lateral loops. Fibrils of the axes and those of the lateral loops may be seen to be continuous. The short loop ends between two homologues of a bivalent appear to line up at the synaptic center to form a continuous dense line. A model of the organization of the synaptinemal complex is proposed, and a photograph of this model is presented.

## ACKNOWLEDGMENT

# TABLE OF CONTENTS

# LIST OF FIGURES

Figure

# KEY TO THE ABBREVIATIONS

ba,  synaptic center
     (or bivalent axis)

Bas, basidium

c,   centriole

ca,  homologous-chromosome axis

Ch,  chromosome

cm,  centromere

ER,  endoplasmic reticulum

f,   fat

Fd,  food particles

G,   Golgi apparatus

Gen, generative cell

Gr,  granules (osmiophilic)

GV,  Golgi vesicles

H,   hilum

ig,  intracisternal granules

Lo,  lomasomes

lp,  lateral loops

M,   mitochondria

N,   nucleus

NM,  nuclear membrane

Np,  nuclear pores

Nu,  nucleolus

P,   septal pore

pf,  chromosome puff

PL,  parallel lamellae

PM,  plasma membrane

R,   ribosomes

S,   septum, or septal swelling

Scp, septal cap

Spz, spindle zone

St,  stalk cell

Stg, sterigma

Syn, synaptinemal complex

V,   vacuole

Vc,  vesicles

W,   cell wall

Note:  Unless otherwise indicated, the scale on the photographs
       indicates 1 micron.

## INTRODUCTION

The fruiting body of <u>Coprinus</u> represents the most
well developed structure in the life cycle of the fungus.
Its morphology, development, and the means by which basi-
diospores are formed and are discharged have long attracted
the attention of many investigators.  Current knowledge of
this fruiting body is the result in large measure of the
work of the late Professor A. H. R. Buller (Buller, 1924,
1931, 1933).

Since the advent of the electron microscope as
a tool for biological research, a number of fine structural
studies with fungal materials have been reported.  Much of
these are concerned with the fungal hyphae.  Similar studies
with fruiting bodies of the basidiomycetes have been scanty.
As far as I am aware, there are only three reports dealing
with fine structures of basidia and basidiospores.  In add-
ition, with a few exceptions, $KMnO_4$ has been used as fixative
which has left out much to be desired, such as the fine
structural organization of the fungal nuclei in general,
and the chromosomes in particular.

In the present investigation, I have made an
attempt to clarify various aspects of micromorphology in
relation to fruit-body development, and particularly, to

obtain information concerning the chromosome structure of
this group of higher fungi. Since the major hiatus of
information on chromosome structure is at the fine struc-
tural level, the use of electron microscopy has been
extensively resorted to, the use of light microscopy be-
ing largely for the purpose of relating fine structure more
definitely with the fungal-cell physiology and cytology dur-
ing fruit-body development.

Because a fruiting body is believed to arise from
a single cell of a hypha, its development into a complex
multi-cellular structure offers a good opportunity for
direct comparison of all cell types and their relation to
cellular differentiation. More importantly, because even
a small fruiting body consists of thousands of basidia in
which meiosis occurs, it may be considered to be very
favorable material for the investigation of the fine
structure of synaptic chromosomes.

Meiosis leading to the production of basidio-
spores, is the prime function of the fruiting body of a fungus.
Since this body, during its development, provides a suitable
micro-environment in which meiosis and spore formation may
take place, the stages of these processes must be related
to fine-structural changes in cellular organelles, for
which the term micromorphogenesis is used in this thesis.

In the present study, the meiotic process was investigated by means of light microscopy. This information was then correlated with electron microscope investigation of cellular organization and development. As pointed out earlier, meiotic stages are indicators of stages of development. How can one identify the meiotic stages in the material being examined with the electron microscope? This identification depends largely on comparison of observations made with the light microscope and with the electron microscope.

The fruiting body of Coprinus offers some advantage in such comparative studies. The basidia mature on the gill from the bottom upwards (Buller, 1931). Thus basidia from about the same position of the fruiting body would be at the same stage of development, or nearly so. Such close synchrony makes the fruiting body convenient material for comparative studies, since half of a fruiting body may be fixed for light microscope observations and the other half for electron microscope examinations.

The nucleus is the least understood of all cellular organelles regarding the fine structure. This obviously is due to the fact that chromosomes are difficult to resolve in ultra-thin sections. In spite of the best technique available, as is pointed out by Ris (1961), the nucleoplasm remains "a monotonous jumble of granules and nondescript fibrils with no evidence of chromonemata, chromosomes or in fact

any continuous structure". The difficulty has been over-
come by the use of whole-mount electron microscopy, which
was first used by Kleinschmidt et al. (1960, 1962) for
bacteria and viruses. This technique has now been widely
applied to animal cells (Ris, 1961, 1962; Ris and Chandler,
1964; Gall, 1963; Wolfe, 1965a; DuPraw, 1964). It is now
well established that fibrils of the order of 100 Å thick
are the structural elements of a chromosome, although how
these fibrils are organized to form a chromosome remains
obscure. Nevertheless, the lampbrush chromosomes in
amphibians (Lafontaine and Ris, 1958) and the metaphase
chromosomes of honey bees (DuPraw, 1964, 1965), as re-
vealed by whole-mount electron microscopy, have shown that
there is definite continuity of the chromatin fibrils.

In the present study, emphasis will be given
to the fine-structural organization of nuclei and chromo-
somes of Coprinus. It is worthy of note that two cell
types of the fruiting body of this basidiomycete are proved
to be of particular interest, namely, the basidium and
the cystidium. In the cystidium, somatic interphase chromo-
somes of its nuclei are resolvable. In the basidium,
the synaptic chromosomes of the diploid nucleus exhibit tri-
partite structure comparable to the synaptinemal complex
of higher plants and animals. Here the synaptic chromosomes
as shown by electron microscopy, is described for fungal

cells for the first time.  The fine structure of such a
complex provides some clues to the interrelationship of
the two homologous chromosomes at synapsis; it also throws
light on the organization of the meiotic chromosomes.

## LITERATURE REVIEW

Much of the work done with fungal materials has been concerned primarily with the organelles of the mycelium (Moore and McAlear, 1961a, 1962a, 1962b, 1963a, 1963b; Moore, 1963c; Girbardt, 1958; Hawker, 1963). Demonstration of the fine structure of fungal chromosomes is lacking. In the present context, I shall review briefly some of the important organelles of fungal cells as they are described in the literature. Pertinent literature will also be referred to later in the discussion. Emphasis, however, will be given to the fine structure of chromosomes in general.

## Fine Structure of Fungal Cells

Electron microscope investigation of fungal cells has revealed that their organization resembles that of higher organisms. Within the cell wall, the protoplast is enveloped by a plasma membrane. The protoplast contains the nucleus, the mitochondria, the Golgi complex, the vacuoles, the ribosomes, and the endoplasmic reticulum. These organelles are embedded in the cytoplasmic ground substance, or matrix (Wells, 1964b, 1965; Moore and McAlear, 1962b, 1963b; Girbardt, 1958; Shatkin and Tatum, 1959).

In most of the work done on fungal materials, $KMnO_4$ has been used as fixative (Moore and McAlear, 1963a; Wells, 1964a). This preserves the membrane systems well, but does not

preserve the structure which contains the nucleic acid.  In
$OsO_4$ fixed materials, however, all structures are preserved
reasonably well (Shatkin and Tatum, 1959; Zalokar, 1961; Luck,
1965a).  The latter fixative is more difficult to use than is the
former, owing to its slow penetration.

Fungal nuclei have not received much attention from
electron microscopists.  This is probably because $KMnO_4$ does not
preserve more than the gross features of nuclear structure.  How-
ever, in $OsO_4$ fixed nuclei, chromatin fibrils about 100 - 150 Å
thick have been shown to be the structural components of the
nucleoplasm (Shatkin and Tatum, 1959; Girbardt, 1958).  It has
been established that the nucleus is enveloped by a double mem-
brane in which there are many pores, or annuli (Moore and McAlear,
1962a; Wells, 1964a).

Electron microscope investigations have shown that the
ribosomes of fungi resemble those of higher organisms (Shatkin
and Tatum, 1959; Zakolar, 1961; Luck, 1965; McManus, 1965).  By
fractionation and differential centrifugation it has been demon-
strated that fungal cells have a ribosomal sedimentation coeffic-
ient of 80 S similar to that of higher organisms (Taylor and
Storck, 1964).

Fungal mitochondria also resemble those of higher
organisms.  This conclusion was drawn by Moore and McAlear (1963b)
after their careful survey of fifty genera of fungi.  These
authors treated the topic thoroughly, comparing fungal mito-
chondria with those of algae, protozoa, higher plants and

animals.  Regarding mitochondria in general, a few excellent
reviews may be found in the literature (Rovillar, 1960; Novikoff,
1961; Lehninger, 1964).

The mitochondria of fungal cells are bounded by a
double membrane, the inner one being folded to form numerous
cristae (Moore and McAlear, 1963b, 1961b; Luck, 1965a, 1965b).
Fungal mitochondria vary in size from 0.5 - 7 micra  long and
in shape from globose to ellipsoidal to elongate, and even
branched (Moore and McAlear, 1963b; Wells, 1965).  The fungal
cristae may be platelets or tubules.  Their arrangement is
random (Moore and McAlear, 1963b, 1961b; Wells, 1964a, 1965).
In yeast cells grown under partially anaerobic conditions, the
cristae may become lamellar (Hirano and Lindegren, 1963).  Within
the mitochondria, particles similar in size and shape to ribosomes
are present (Luck, 1965a, 1965b; Shatkin and Tatum, 1959).  Fungal
mitochondria also contain DNA (Buck and Luck, 1964).

The endoplasmic reticulum is prominent in fungal
cells.  In thin sections, it may have the appearance of a system
of circular or longitudinal cisternae (Moore, 1963a; Wells, 1964a;
Berliner and Duff, 1965; Moore and McAlear, 1962a).  The diameter
of the circular cisternae is 55-140-800 m$\mu$, but measurement of
the distance between two membranes of longitudinal cisternae
yields values of 20-75 m$\mu$, comparable to the distance between
the two membranes of the nuclear envelope.  Moore (1963a) con-
sidered the latter measurement a correct evaluation of tubule
diameter of the endoplasmic reticulum.  The endoplasmic reticulum

is found to be continuous with the nuclear membrane (Moore and
McAlear, 1962a; Wells, 1965), with the plasma membrane (McAlear
and Edwards, 1959; Moore, 1963a; Hashimoto et al., 1960), and
with the mitochondrial membrane (Berliner and Duff, 1965; Moore,
1963b). McAlear and Edwards (1959) suggest that the connection
of the plasma membrane and the nuclear membrane with the endo-
plasmic reticulum lends support to the general concept of an
evolutionary sequence in the development of the nucleate from the
anucleate cell. It was observed that the endoplasmic reticulum
may fold and become lamellar (Wells, 1964a) or may form a con-
centric complex (Berliner and Duff, 1965; Moore, 1963a). The
function of the lamellar endomembrane and the concentric complex
is not known.

The Golgi dictyosome in fungi was first demonstrated
by Moore and McAlear (1962b, 1963a) in Neobulgaria pura, a dis-
comycetous fungus. Later this organelle was observed in the
phycomycete Pythium debaryanum (Hawker, 1963), in the heterobasid-
iomycete Puccinia podophylli (Moore, 1963c), and in the myxomycete,
Hemitrichia vesparium (McManus, 1965). Their demonstration was
questioned by Wells (1964a) who expressed doubt that the parallel
lamellae he observed can be equated with the Golgi dictyosome of
animal cells, but, in view of the evidence of other workers, the
presence of a Golgi complex in fungi appears to be a reality.

The Golgi dictyosome of fungi resembles that of higher
plants and animals in having a stack of sacs from whose margins,
vesicles are "blebbed" off. Like animal cells, and unlike

plant cells, this body lies in close proximity to the nucleus
(Moore, 1963c).  From most published electron micrographs, the
Golgi dictyosome appears to be about 0.5 micron, long (Moore
and McAlear, 1963a; McManus, 1965).  The demonstration of an
expanded Golgi dictyosome (about 4 microns long) in cells of
the aecial primordium of <u>Puccinia</u> <u>podophylli</u> is exceptional
(Moore, 1963c).  The variation of this organelle in relation to
development remains to be investigated.

There are two structures which are noteworthy char-
acteristics of fungal materials, namely lomasomes and septal
pores.  The lomasome was first named by Moore and McAlear (1961a).
They claimed that this was a "previously uncharacterized hyphal
structure".  However, such structures were described by Girbardt
(1958) as "rings" located outside the plasma membrane.  Moore
and McAlear (1961a) stated, that they "have failed to find them
(lomasomes) in  conidia, ascospores, basidiospores, or the cells
from which these arise".  Later work by Wells (1965) gave
evidence that lomasomes are present in the basidium.

The function of lomasomes is not known.  Girbardt (1958)
suggests that vesicles seen in lomasomes may be the plasma pore,
or that they may be invaginations of the plasma membrane.  Moore
and McAlear (1961a) suggest that vesicles originating within
the cell move out and fuse with the plasma membrane, releasing
substances to the outside of the protoplast.  They further
suggest that lomasomes may be involved in cell-wall metabolism.
On the other hand, Girbardt (1958) finds that the "rings" are

larger in older cells than in young ones.  This observation
implies that lomasomes are related to the ageing of cells.

The septal pores of fungal cells are unique.  The
plasma membrane is actually continuous from cell to cell through
the septal pores (Berliner and Duff, 1965; Moore, 1963a, 1963b;
Wells, 1964a; Giesy and Day, 1965; Reichle et al., 1965; Moore
and McAlear, 1962c).  This makes the fungal mycelium essentially
a coenocytic system, even though a particular "cell" may have
only one or two nuclei.

The septa of ascomycetes and of the rust fungi have
simple pores (Moore, 1963a, 1963b; Reichle, et al., 1965; Shatkin
and Tatum, 1959).  The septum may be formed centripetally by the
extension of the cell wall (Buller, 1933; Moore, 1963b); however,
the exact manner of septum formation is not clearly understood.
As Moore (1963b) suggests, there are two possible ways in which
the septum could be formed:  "In one the lip or front of the
invagination-wedge would be the source of new wall material and
as a consequence the septum would be composed of homogeneous
wall material coherent with the outer hyphal wall.  In the
second the closely opposed membranes would each be the prime
producers of wall material and this would consequently produce
two closely appressed noncoherent transverse walls that topo-
logically would be an infold extension of the outer wall."
There may be a single septal pore or several (Reichle et al.,
1965).  Nuclei and other organelles can stream through the
pore easily (Shatkin and Tatum, 1959).

The septal pores of homobasidiomycetes are of particular interest. They have a rather complex and unique structure. Around the pore, the septal wall swells considerably. The material that constitutes the septal swelling is different from the wall material (Berliner and Duff, 1965; Bracker and Butler, 1963). Over the pore, there is on each side a septal cap which has been termed "parenthesome" by Moore and McAlear (1962c). In thin sections, the septal cap membrane is observed to be discontinuous (Wells, 1964a; Moore and McAlear, 1962c; Berliner and Duff, 1965; Giesy and Day, 1965). It is multilayered and is claimed to be continuous with the endoplasmic reticulum (Berliner and Duff, 1965).

It would seem that such a septal cap might not permit free streaming of the nuclei. However, a very interesting observation has been made recently by Giesy and Day (1965). During nuclear migration, the septal swelling and the septal caps are dissolved and the septum becomes a simple pore, like that of ascomycetes. This important discovery has advanced the current knowledge of the mechanism of nuclear migration in these basidiomycetes.

## Chromosome Structure

Since the rediscovery of Mendel's work in 1900, the science of genetics has progressed at an ever-accelerating pace. Shortly after 1900, Sutton (1903) brought out the chromosome theory to explain the genetic mechanism. This was later supported and confirmed. Morgan (1910) established that a gene

may be located on a particular chromosome, and Sturtevant (1913)
showed that genes are arranged in a linear sequence along a
chromosome.  Creighten and McClintock (1931) demonstrated that
cytological crossing-over and exchange of chromosomal sequence
is accompanied by the expected gene exchange.

It is now well established that the chromosome is a
carrier of genetic materials.  Needless to say, the understand-
ing of chromosome structure is of paramount importance to the
understanding of the mechanism of crossing-over.  Conversely,
the knowledge of crossing-over undoubtedly offers clues regard-
ing the structure of a chromosome.

However, despite extensive research, neither the pro-
blem of chromosome structure nor the problem of the mechanism
of crossing-over has been completely solved.  Advances have
been made and evidence has been accumulating; nevertheless, the
current treatment of chromosomal fine structure remains in the
domain of models and hypotheses.

Since the advent of the electron microscope as a tool
for biological research, the structure of cytoplasmic subcellu-
lar organelles has been elucidated.  However, similar studies
of the nucleus have proved to be unrewarding (Ris, 1961; Swift,
1962).  This may be because (1) it is difficult to identify the
division stage of a chromosome in a sectioned nucleus, (2) the
entangling of chromosomes in a nucleus obscures the identity of
an individual, or (3) the fibrillar continuity in ultra-thin
sections is disrupted.

More recently, whole mounts of isolated chromosomes
have been prepared by monolayering isolated nuclei on an air-
liquid interphase (Kleinschmidt et al., 1960, 1962). The pro-
cedure was applied successfully to sea urchin sperms (Solari,
1965), and to the embryo cells of honey-bees (DuPraw, 1964, 1965).
It has also been used for other organisms (Gall, 1963; Ris, 1961,
1962). The information provided by whole-mount chromosomes
coupled with the study of sectioned materials may eventually
lead to a solution of the problem of the structure of a chromo-
some.

## 1. The Basic Structure of a Chromosome

It is well established that a chromosome consists of
DNA, RNA, histones, and residual proteins (Brachet, 1957; Osawa
and Mirsky, 1959; Busch et al., 1964). The DNA macromolecule,
the carrier of genetic information, is the most important com-
ponent of a chromosome. In the currently accepted view, it is
in the form of a Watson-Crick double helix (Watson and Crick,
1953). The association of the DNA double helix with other
chromosomal components, namely histones and the residual pro-
teins, constitutes a chromatin complex which is the structural
material of a chromosome. The molecular organization of these
components into chromatin microfibrils has been described in
a recent review (Ris, 1961). This complex, as revealed by the
electron microscope in both sectioned and whole-mount prepara-
tions alike, exhibits a unique fibrillar structure. The fibrils
are extremely long and entangled; in whole-mount preparations,
as in DNA-histone extracts, few free ends may be seen (Wolfe,

1965a; DuPraw, 1964; Ris and Chandler, 1964).

The width of the chromatin fibrils is remarkably consistent from species to species, although there are slight variations. As an example of such variations in whole mount preparations, microfibrils 200-250 Å thick were reported in grasshopper (Dass and Ris, 1958), honey bee (DuPraw, 1964) and salamander (Wolfe, 1965a; Lafontaine and Ris, 1958). Micro-fibrils 400 - 600 Å thick in the newt, Triturus were reported by Gall (1963). Wolfe (1965a) suggests that unusually thick fibrils are the result of contamination. Fibrils of the sal-amander 600 Å thick were also reported by Lafontaine and Ris (1958) when isolated chromosomes were fixed with ethanol. These authors state that, after ethanol fixation, fibrils are usually clumped in pairs with the result that the unit fibrils as revealed by the electron microscope are 500 Å thick. Thus the fibrils 500 Å thick observed may not be natural.

In sectioned materials fixed in OsO$_4$ or formalin, fibrils 100 Å to 150 Å thick have been observed in a variety of materials, including microsporocytes of Tradescantia, spermatids of Octopus vulgaris, spermatids of rooster, spermatids of Ranatra spp, calf thymus nuclei (Ris, 1961), spermatocytes of Triturus (De Robertis, 1956) and somatic nuclei of Neurospora crassa (Shatkin and Tatum, 1959).

Variation in the thickness of chromatin microfibrils within a cell type has also been noted. De Robertis (1956)

measured a good many samples of the microfibrils of early prophase, later prophase and metaphase of meiosis in <u>Triturus</u>. He finds that the microfibrils are thicker at metaphase than at prophase. In early prophase, they have a mean thickness of 47 Å. In late prophase, the mean thickness is in the neighborhood of 70 Å, and in metaphase, they vary from 60 - 170 Å with a mean at about 100 Å thick. Wolfe (1965a) finds, however, that the chromatin fibrils of metaphase chromosomes isolated from cultured bovine kidney cells are identical in their thickness to those of the interphase nuclei of bovine cells as well as of amphibian cells. Similar results were obtained by DuPraw (1964) with embryo cells of the honey bee. Undoubtedly, variation in diameter of the microfibrils may occur, but variation so well patterned as that reported by De Robertis should be accepted with caution. Further investigation is needed.

Whether the chromatin microfibril is made up of a single strand, or of a bundle of nucleoprotein strands is controversial. Ris and his colleagues (Ris, 1961, 1962; Lafontaine and Ris, 1958; Dass and Ris, 1958; Ris and Chandler, 1964) contend that the fibrils of the chromosome are made up of a bundle of subfibrils. Gall (1963), Wolfe (1965a), and DuPraw (1964), however, denied the existence of subunits.

Ris and his colleagues claim to have observed a fibril 250 Å in thickness which is made up of two 100 Å subunits, each probably consisting of two 40 Å nucleoprotein fibrils. Ris and Chandler (1964) claim that when the chromosome fibrils of

erythrocyte nuclei of <u>Triturus</u> <u>viridescens</u> are prepared in water,
they are 200 - 250 Å thick; but when they are prepared in a sol-
ution containing 0.07 M NaCl, 0.001 M versene, ammonium acetate
and cytochrome-c at pH 8, most of the 200 Å fibrils are separated
into 100 Å fibrils.  They further claim that the 100 Å threads
consist of two subunits about 40 Å thick.  In supporting their
contention, they treated the 200 Å fibrils with 0.2 N HCl for 30
min to remove the histones.  They presented photomicrographs in
which they claim that both 200 Å and 40 Å fibrils are seen; there
is no intervening 100 Å threads.  Unfortunately, there are no
evidence that the 200 Å fibrils actually separate into two 100
Å threads, nor 100 Å threads into two 40 Å fibrils.

On the contrary, chromatin fibrils, 200 - 250 Å thick,
have been claimed to be single, as reported by Wolfe (1965a) and
Gall (1963) in <u>Triturus</u>, by DuPraw (1964, 1965) in honey-bees.
The best evidence supporting this view was provided by DuPraw
(1964) who digested the chromatin fibrils with trypsin.  The
trypsin-digested fibrils show a long trypsin-resistant (yet
DNase-susceptible) core which has a diameter as small as 23 - 26
Å.  DuPraw (1965) argues that these dimensions are consistent
with an interpretation of the core as a single Watson-Crick
DNA molecule.  This worker presented an electron micrograph
of a long chromatin fibril unevenly digested by trypsin; this
was considered as evidence that a 250 Å fibril contains only
one 20 - 30 Å DNA molecule.

Since Ris and Chandler (1964) and Wolfe (1965a) both studied microfibrils of the same organism, <u>Triturus viridescens</u>, a closer re-examination and comparison is desirable.  Judging from the electron micrographs published by these authors, one cannot infer any substantial difference in the fine structure of the chromatin microfibrils.  As pointed out by Wolfe (1965a) and DuPraw (1965), the microfibrils are "bumpy".  The bumpiness is taken by Ris and Chandler (1964) to mean twisting of two sub-units, whereas the same bumpiness is considered a mere twisting of the fibril itself by DuPraw (1965).

The lampbrush chromosomes of the amphibian are giant chromosomes which are particularly useful for study of their organization.  Details concerning these chromosomes may be found in a recent review (Swift, 1962).  They appear to have many lateral loops radiating from the chromosome axis.  It is generally believed that the loop is a part of the chromonema  which runsthroughout the entire length of the chromosome (Swift, 1962; Ris, 1961; Lafontaine and Ris, 1958).  As pointed out by Nebel and Coulon (1962a), the lampbrush type of chromosome may also occur in other cell types, although on a miniature scale.  If this is true, the loop of the giant chromosomes of the amphibian is comparable to the chromatin fibrils of other cell types.

In summary, one can only say that the information currently available concerning strandedness of chromatin fibrils is insufficient to allow one to draw a definite conclusion.  The disparity seems to lie in the interpretation of what may be seen

by workers with the electron microscope. However, the concept

that the fibril is a nucleoprotein complex involving a single

DNA double helix may be more acceptable, judging from DuPraw's

recent investigation, although this requires further confirmation.

As DuPraw (1965) visualized it, this complex is made up of a

central core of DNA surrounded by a trypsin-susceptible sheath

of distinctly different physical and chemical properties. This

complex is the basic structure of a chromosome.

## 2. The Organization of Chromosomes

The question is now raised as to how these microfibrils

become organized into the chromosome which has been recognized

for so long by light microscopists. Under the light microscope,

individual chromosomes exhibit a definite pattern, although they

may appear smooth or coiled. In contrast, electron microscope

studies of chromosomes in thin sections do not seem to provide

information beyond revealing masses of microfibrils which can,

at best, be suggestive with respect to the organization of a

chromosome. This may account for the controversy about the organ-

ization of a chromosome.

Since the macromolecular architecture of the genetic

material, DNA, proposed by Watson and Crick (1953) has now been

generally accepted, several models have been proposed for the

organization of the DNA molecules in a chromosome (Freese, 1958;

Taylor, 1957, 1964; Schwartz, 1958; Ris, 1961). The models are

classified by Swift in his recent review (1962) as follows:

(1) protein-backbone theories; (2) the multistrand or "rope"

theories; and (3) differential-coiling theories. Some of the models are mainly concerned with the molecular organization of the DNA in a chromosome; others are also concerned with the physical organization of the chromatin fibrils into a chromosome. In the present review, only the latter models will be discussed in detail.

Ris and his colleagues (Ris, 1961, 1962; Dass and Ris, 1958; Lafontaine and Ris, 1958; Ris and Chandler, 1964) favor the multistrand hypothesis. This view is also shared by Kaufmann et al. (1960) and by Steffensen (1959). Ris (1961) claims that there are 32 fibrils in a Tradescantia chromosome. On this assumption, he proposes a model in which a bundle of nucleohistone fibrils constitutes the chromosome axis. Each of these fibrils is individually coiled. These fibrils become joined in a super- coil, and this gives the chromosome its recognizable form. Evidence which Ris (1961) adduced in support of the multistrand model is the following: (1) direct observation under the light microscope shows that anaphase chromosomes are double; (2) X-ray breakage reveals subchromatids; (3) sea urchin eggs which have been inhibited by mercaptoethanol can divide directly into four nuclei with a four-polar spindle if the inhibitor is removed at the time when the control eggs are undergoing second division. This is an unpublished experiment by Bibring reviewed by Mazia (1960). Further details regarding the multistrand hypothesis may be found in recent reviews (Ris, 1961; Kaufmann et al., 1960).

More recently, Wolfe (1965b), studying specially prepared whole-mounts of cultured bovine cells, chooses to accept the multistrand concept of a chromosome. He suggests that the multistrandedness may occur either at the level of the whole chromatid or of the fiber core, although he does not agree with Ris and Chandler (1964) on the nature of the chromatin fibrils (he states that the chromatin fibrils are single). Wolfe's suggestion is not accepted by DuPraw (1965).

The multistrand model proposed by Ris has not gained general acceptance by geneticists. As Schwartz (1960), Taylor (1964) and DuPraw (1965) have pointed out, the concept of a multistranded chromosome imposes many difficulties. Ris (1961) admits this shortcoming but he argues, "it will be more productive to try to understand chromosome mechanics in terms of a multistrand model and to look into the possible evolutionary advantages of a multistranded chromosome than to make believe that the chromosome has the properties of a single DNA molecule".

More recently, DuPraw (1964, 1965) studies honey-bee metaphase chromosomes by means of whole-mount electron microscopy. He finds that in a metaphase chromosome the chromatin microfibrils are folded irregularly. On the basis of this evidence, DuPraw (1965) proposes a folded-fiber model. The concept of this model may be stated as follows. As in bacteria and viruses, the DNA occurs as a relatively small number of single and very long Watson-Crick molecules. Each DNA molecule is

surrounded by a protein sheath, leading to one-to-one corres-
pondence between the observed 230 Å fibril and a single DNA
molecule (which constitutes the fiber core). In mitosis, the
protein-sheathed DNA molecules fold up or are folded, in re-
producible patterns. This folding is irregular in the sense
that the gross structure of the mitotic chromosome need nei-
ther be linear nor show any direct similarity to the organi-
zation of the DNA molecule. During interphase, the different
DNA molecules (and corresponding fibers) constitute indepen-
dent functional units, both in RNA synthesis and in DNA repli-
cation. This implies that replication proceeds sequentially
along each fiber at a DNA "replication fork" and it also im-
plies that RNA "read out" is controlled at the level of the
single DNA molecule. DuPraw (1965) suggests that the cen-
tromere constriction may contain a final short section of un-
replicated DNA which could be partly or entirely responsible
for keeping the chromatids together until anaphase. This
folded-fiber model, DuPraw states, "is compatible with the
basic experimental results pertaining to chromosomes and nuclei
in general".

Detailed information about this model may be found
in DuPraw's paper (1965). Only a few key points will be men-
tioned here. For example, it is compatible with the semi-
conservative mode  of DNA replication of the Watson-Crick
model. It requires that short-term incorporation of tritiated
thymidine must be approximately symmetrical in the two chrom-
atids. This pattern of replication of the DNA has been sup-

ported by autoradiographic investigations (see DuPraw, 1965;
Hsu, et al. , 1964, for reviews).

It also implies some specific restrictions. One of
these is that the labelling of chromosomes by DNA precursors
cannot be synchronous, but is instead localized; the telomere
region is labelled first; and the centromere region last. An-
other implication is that the DNA content will be proportional
to chromosome volume. Short chromosomes complete replication
first as has been borne out by several investigations (German,
1964; Lima-de-Faria, 1959).

Gall (1958) proposes the idea that a chromonema is
made up of a singel nucleohistone fiber coiled differentially
to form a chromosome. In lampbrush chromosomes, there are chrom-
mere regions as well as interchromomere regions. These two
are linked linearly to form the chromosome axis from which
loops project laterally. Gall considers that the chromonema,
which is continuous throughout the whole chromosome, is coiled
tightly in the chromomere region.

In the foregoing paragraphs, several models visual-
ing chromosome organization have been reviewed briefly. Now
it may be appropriate to consider the acceptability of each of
these. In a recent review, Swift (1962) has discussed and
appraised the chromosome models proposed. However, a few com-
ments need to be made, because more evidence has now become
available. The multistrand model poses considerable difficulty
in relation to the known facts of genetics. Besides, all

experimental evidence in support of this hypothesis can be accommodated by the fact that there may exist a "half-chromatid". However, the existence of a half-chromatid in one species does not necessarily mean the existence of the same in all species.

The folded-fiber model of chromosome structure appears to be the most acceptable of the models proposed. It is compatible with known genetic and cytological processes. It is the simplest model and yet is sufficient to explain and fit in with most observations. However, even here, there are minor difficulties. For example, the model suggests that the centromere is not replicated until metaphase-anaphase. This notion is not compatible with autoradiographic studies which show that no labelling by isotope occurs at metaphase or even at late prophase. It is also not compatible with the finding that the centromere is already divided at metaphase (Lima-de-Faria, 1958). The model also suggests that the contractile protein sheath may be responsible for chromosome contraction. When the sheath is removed by proteases, the DNA core springs out instantly. Undoubtedly the proteins associated with DNA may be responsible for the coiling or folding of the DNA-protein fibrils. Whether or not the proteins really form a sheath is still obscure.

To summarize this section on chromosome organization, I agree with Swift in his conclusions. These are (1) that a chromosome may contain a single DNA helix or possibly as many

as two helices running throughout its length, and (2) that differences between the chromosomes of different species may possibly occur.  Furthermore, the single nucleohistone fiber may undergo secondary coiling.  There is no tertiary or quaternary coiling.  Rather, the chromatin fibers fold irregularly to form a reproducible configuration during chromosome condensation at metaphase.

3. The Organization of a Synaptic Chromosome at Meiosis

In contrast to mitotic chromosomes which do not show any organizational pattern (at least as revealed in thin sections under the electron microscope) the fine structure of a meiotic chromosome exhibits a particular organization pattern which is now known as the synaptinemal complex, which occurs only when the nucleus is at the synaptic stage, i.e. zygotene to pachytene. This synaptinemal complex was first demonstrated by Moses (1956) in crayfish.  Its existence was denied by De Robertis (1956), but was later supported by Fawcett (1956) and has now been established in the pigeon, cat, and man (Fawcett, 1956), in the grasshopper (Moses, 1958), in Tradescantia and Lilium (Ris, 1961), in the domestic rooster (Coleman and Moses, 1964) and in several other organisms (Nebel and Coulon, 1962a; Sotelo, et al., 1960,  1964). These researches have been reviewed recently by Moses and Coleman (1964).

There is general agreement that this complex is of tripartite structure or at least double.  It consists of two outer or lateral axial elements and a central one.  The size of

these elements varies somewhat from species to species. In general, the two lateral axial elements are about 300 - 450 Å thick, and the central one about 120 - 170 Å thick. The lateral axial elements are separated by 600 - 1200 Å (Moses, 1958; Fawcett, 1956; Nebel and Coulon, 1962a; Moses and Coleman, 1964). Moses (1956) postulated that the synaptinemal complex is cylindrical, while Fawcett (1956) suggested that it is a ribbon-like structure with its margins formed by two dense parallel fibrils evenly spaced on either side of a third slender electron-dense line. The complex is sharply defined and it stands out clearly against a background of karyoplasm of very low electron density (Fawcett, 1956). Similar observations were made by Moses and Coleman (1964) and Nebel and Coulon (1962a).

In a view of frontal sections, the three elements are seen to lie parallel in the same plane. The two lateral axial elements are mirror images of one another, the central element lying between them (Moses, 1958; Moses and Coleman, 1964; Fawcett, 1956; Nebel and Coulon, 1962a; Sotelo et al., 1964). This complex is apparently twisted along its long axis (Moses and Coleman, 1964; Nebel and Coulon, 1962a). The bulk of the chromatin fibrils are arranged in association with the two lateral axial elements. The chromatin masses vary in size and shape along the chromosome. These, according to Moses and Coleman (1964), are usually bilaterally symmetrical and probably correspond to the chromomeres seen after acid fixation and in squash preparations.

        The fine structure of the complex is not understood
completely, owing to the limitations of resolution. In electron
micrographs, the lateral elements appear to be two parallel
electron-dense lines from which microfibrils radiate laterally
(Nebel and Coulon, 1962a). In favorable sections, these lines
appear to be composed of tightly packed microfibrils that are
continuous with the chromatin masses (Moses and Coleman, 1964).
Whether the microfibrils are free-ending extensions (bristles)
from the axial elements, or are loops has not been determined
(Moses, 1960; Nebel and Coulon, 1962a).

        The central element is  first described by Moses
(1956, 1960) as a third fibrillar component. This interpretation
is  questioned by Fawcett (1956) who then suggests that it may
be merely a condensation of material at the interphase between
two cylinders of karyoplasm organized around the two larger
lateral fibrils. Moses and Coleman (1964) later agree with
this interpretation of Fawcett in view of the fact that this
central element is transient. Furthermore, they suggest  that
the central line may be an area of effective pairing of homologous
chromosomes (Moses and Coleman, 1964).

        In transverse sections, the synaptinemal complex is
revealed as three dots in a row; a small central one, flanked
by two larger ones (Fawcett, 1956; Nebel and Coulon, 1962a).
The central element may appear as a short electron-dense line
(Sotelo, et al., 1964), and the lateral axial elements may be
revealed as a single dense spot about 600 Å in diameter, or as

the two chromatids of a homologous chromosome (Nebel and Cou-
lon, 1962a).  Between the two lateral elements (the homologues)
are fibrils which are oriented in such a way as to suggest
that they connect the homologous chromatids (Nebel and Cou-
lon, 1962a; Moses and Coleman, 1964).

Although questioned by some workers, an equation of
the synaptinemal complex with synaptic pairing in meiosis is
at present favored by the majority of workers in the field.
Moses (1958) suggests that the structure must be regarded as
a phenomenon of meiosis and specifically of the phase in which
chromosome pairing occurs.  The evidence in support of this
concept is that this complex has not been observed in presyn-
aptic, in post-synaptic, and in any other meiotic stages or in
mitosis (Moses, 1958; Moses and Coleman, 1964; Ris, 1961; Nebel
and Coulon, 1962a).  Moses and Coleman (1964) conclude , there-
fore, that this complex is a part of a bivalent chromosome and
exclusively associated with synapsis.

This hypothesis has been questioned by Sotelo and
colleagues (Sotelo et al., 1960; Sotelo, 1962).  They present
electron micrographs of Gryllus cells in which five or six para-
llel lines are seen.  These authors suggest that the tripartite
core (synaptinemal complex) represents a homologous chromosome.
This view is not supported by observations of other workers.
The multiple parallel lines (axial elements) seen by Sotelo
(1962) may be considered instead as multiple association
of bivalents.  More recently, similar multiple parallel

lines have been observed in _Gryllus_ by Schin (1965). This author presents electron micrographs revealing that these parallel axial elements are arranged concentrically around the nucleolus. This suggests that the multiple parallel lines are the result of multiple association of chromosomes at the early synaptic stage when polarization of chromosomes occurs as such reported by Taylor (1950).

The best evidence supporting Moses' hypothesis so far available is the observation of Meyer (1960, 1964), who finds that, in comparative studies of _Drosophila_ oocytes and spermatocytes, the synaptinemal complex has been observed abundantly in the oocytes but has not been observed in the spermatocytes. Although meiosis does occur in the spermatocytes of _Drosophila_, crossing-over does not occur between homologous chromosomes. Interestingly enough, in a _Drosophila_ mutant in which synapsis and crossing-over are suppressed, the synaptinemal complexes cannot be found even in the oocytes (Meyer, 1964). Further, in _Tipula_ _oleracea_ and _Phryne_ _fenestralis_, which are achiasmatic, synaptinemal complexes are not found, while in a chiasmatic species of _Tipula_, they are. If Meyer's observations are correct, they suggest strongly that the synaptinemal complex is not only characteristic of a bivalent, but also is strictly a synaptic device which is involved in crossing-over.

In the early phase of synapsis, pairing between homologous chromosomes occurs. If one cuts a section through a paired bivalent, one gets a synaptinemal complex. On the

other hand, if one cuts a section through an unpaired chromosome, one gets a single chromosome structure. Nebel (1959) claims that the frequency of the single element decreases as pairing continues, while the frequency of the complex increases. Meyer (1964) also shows that in triploid _Drosophila_ females, where synapsis occurs between pairs and leaves one member unpaired, synaptinemal complexes and single structures are observed in close proximity, suggesting that the complex occurs in paired segments and the single structures in unpaired ones. Observations such as these, if confirmed, may lend further support to the concept that the synaptinemal complex is indeed a part of a bivalent chromosome associated with synapsis. However, one should be cautioned that the single structure seen by Nebel (1959) and by Meyer (1964) may be a view of the longitudinal section through a single homologous chromosome of the bivalent.

In summary, one may conclude that the synaptinemal complex is axial to a bivalent chromosome and may be closely related to the process of crossing-over.

Although the existence of the synaptinemal complex has been well established, the organization of the structural elements (the chromatin microfibrils) into a chromosome remains a problem. Two models have to date been proposed by Nebel and Coulon (1962a) and by Moses and Coleman (1964) respectively to explain observations made using the electron microscope. Nebel and Coulon's model is merely an interpretation of the electron micrograph; it does not explain how the chromatin fibrils are

organized in the synapsing chromosomes. In this model, four cylindrical vertical columns are envisaged as representing the four chromatids. Each pair of chromatids is contained in a cylinder which is ellipsoidal in transverse sections. The cylinder with its two chromatids represents the lateral component as revealed by the electron microscope. Between the vertical column is the mid-axial space. The latter is approximately rectangular in transverse view and is subdivided by fine striations. Lateral loops are inserted in the lateral component.

The model of Moses and Coleman (1964) seems to accord better with actual observations. Firstly, their diagram is recognizable as a bivalent chromosome, although it is admittedly no more than an interpretative drawing. These authors state that the main elements are each axial to a homologue and that, when two homologous chromosomes pair, the elements pair, moving toward the central axis of the bivalent. The central element is presumed to be formed as a consequence of pairing. Secondly, their model provides a suggestion as to how the chromatin fibrils may be organized to form a chromosome. The authors are in favor of the lampbrush hypothesis of Gall (1958). They suggest that the microfibrils emanating from the axis are actually loops. The axis thus consists of two chromatids, each composed of a chain of microfibrils joined by hypothetical links (possibly protein) and looped out laterally.

One can agree with Moses and Coleman (1964) that the study of a meiotic chromosome by means of thin-section techniques

is insufficient to allow reconstruction of a perfect model of

the organization of a synaptic bivalent. The information so far

obtained by means of thin-section techniques is nevertheless of

value. Such information when coupled with that obtained by

whole-mount techniques may eventually reveal some important clues

as to the actual organization of a synaptic chromosome.

## MATERIALS AND METHODS

Two species of Coprinus, C. lagopus and C. comatus,
were chosen for the present investigation.  Cultures of Coprinus
lagopus were supplied through the kindness of Dr. Leslie K. Crowe
of the Botany School, Oxford University, England.  Fruiting
bodies of C. comatus were collected from Edmonton gardens.
Dikaryotic mycelia of C. lagopus were subcultured on Brodie's
agar medium (Brodie, 1948) and were photoinduced to fruit.  Young
fruiting bodies of C. lagopus and C. comatus in various stages
of development were dissected for fixation and subsequent treat-
ment.

The genus Coprinus is one of the largest and the
most heterogeneous of the fungi of the family Agaricaceae.  The
species of Coprinus are characterized by autodigestion of the
fruiting body when spores are ready for discharge.  Autodigestion
takes place from the bottom of the gills upward (Buller, 1931).
Coprinus is universally distributed and is one of the most widely
investigated genera regarding morphology, development (Buller,
1924, 1931, 1933), sexuality (Buller, 1931) and genetics
(Papazian, 1958; Day and Anderson, 1961; Day, 1960, 1963a, 1963b).

Coprinus lagopus Fr. is a coprophilous species
commonly found in North America.  Its sexuality is of the tetra-
polar pattern.  The interactions of haploid mycelia are controlled
by two series of incompatibility factors, the A and the B

series (Day, 1960, 1963a; Fincham and Day, 1963).  As far as
is known, each of these series has two loci, $\alpha$ and $\beta$, with a
series of multiple alleles at each locus (Day, 1963a, 1963b;
Fincham and Day, 1963).  Coprinus comatus Fr. is a bipolar
species generally found on garden soil.

The life cycle of Coprinus lagopus is similar to that
of other Hymenomycetes.  Basidiospores are haploid and generally
binucleate.  Upon germination, a basidiospore gives rise to a
haploid (or homokaryotic) mycelium, which bears oidia for asexual
propagation.  When two compatible homokaryotic mycelia meet,
plasmogamy takes place to give rise to a dikaryotic mycelium.
The dikaryotic mycelium is characterized by clamp-connections,
binucleate cells, acute-angle branching between a hypha and the
main mycelium, and by the absence of oidia.  This dikaryotic
mycelium is able to fruit when it receives the necessary photo-
induction.  The fruiting body bears gills upon which basidia
and cystidia are produced abundantly.  In the basidium, nuclear
fusion occurs, followed by meiosis leading to the production of
four basidiospores.  The cystidia act as "pillars" to keep two
adjacent gills apart.  Cystidia disintegrate after the basidio-
spores have been discharged.

For light microscopy, fruit bodies were fixed in Lu's
BAC fixative (9 parts of butanol, 6 parts of glacial acetic
acid, 2 parts of 10% aqueous chromic acid, Lu, 1962).  The
technique was essentially the same as that described earlier
(Lu and Brodie, 1964) except for hydrolysis.  Each fixed fruit-
ing body was dissected into small pieces and hydrolyzed in HCl-

ethanol solution (1 part of 6N HCl and 1 part of 95% ethanol)
in a small Petri dish. The solution with the fungus material
in it was heated to almost boiling over a gas burner. It was
removed from the heat and the material was allowed to hydrolyze
in the solution for $1\frac{1}{2}$ - 2 min. The material was washed thor-
oughly in Carnoy's fixative (6 parts of ethanol, 3 parts of
acetic acid, 3 parts of chloroform) for 5 - 10 min. A small piece
of a single layer of gill was then removed and squashed in propion-
ocarmine. The preparation was examined with a Leitz Ortholux
II microscope.

For electron microscopy, fruiting bodies of C. lagopus
were removed individually from a culture dish, and were carefully
cut in half longitudinally. One half was fixed in Lu's BAC
fixative (see above) and squashed in propionocarmine as described
above. The stage in the meiotic process was then determined by
light microscopy. The other half was carefully cut into 0.5 mm
squares and fixed for 2 hr in Kellenberger's fixative (1% $OsO_4$
in Kellenberger's buffer at pH 6.7) (see Kay, 1961). The fixa-
tive was stored in a refrigerator at $4^{\circ}$ C. During fixation, its
temperature was allowed to rise gradually to room temperature.
The fixed material was washed for 2 hr in Kellenberger's buffer
to which uranyl acetate was added to make a 0.5% solution. The
material was then dehydrated in an ethanol series (30, 50, 70,
80, 90, 100%), a time interval of 30 min. between changes being
allowed. After two changes in 100% ethanol, the material was
then embedded in araldite mixture (1 part araldite + 1 part
dodecenyl succinic anhydride + 0.5 - 1% N-benzyldimethylamine

as accelerator).  The embedding schedule is as follows:

    2 parts ethanol + 1 part propylene oxide .......... 10 min.

    1 part ethanol + 2 parts propylene oxide .......... 10 min.

    pure propylene oxide (2 changes) ................... 15 min.

    1 part propylene oxide + 1 part araldite mixture .. 2 hr -
                                              overnight

    araldite mixture in gelatin capsule No. 00 ........ overnight

The materials in gelatin capsules were subjected to vacuum to

remove trapped air.  Polymerization was then allowed to take

place at 55 °C.  Ultra-thin sections were cut with a Porter-Blum

ultramicrotome Model MT-II using a glass knife.  Sections were

picked up on 150-mesh copper grids and were examined with a

Philips electron microscope Model 100B using an accelerating

voltage of 60 Kv.  Photographs were taken with Kodak fine-grain

positive film, emulsion Kodak p4-26-1, and developed in Kodak

D19 developer.

OBSERVATIONS

Fine Structure of Subcellular Particles in Cells of Coprinus

1. The Golgi Apparatus

The Golgi apparatus may be closely associated with the nucleus in the basidium of Coprinus lagopus or it may not (Figs. 1,2,3). At meiotic prophase I, this organelle was always detected in the upper (or apical) quarter of the basidium (Fig. 1).

The Golgi complex is characterized (in thin sections) by a (presumably) lipid-rich central area from which many cisternae radiate. The cisternae appear like finger prints radiating in one direction (Figs. 2,3) or in all directions (Figs. 4,5). From the study of favorable serial sections, it appears that the Golgi apparatus of Coprinus is probably a closed system (as shown in Figs. 4, 5). This system is represented diagrammatically in Fig. 5a. It consists of a stack of linked, flattened saucer-like sacs.

Unlike the endoplasmic reticulum, the cisternae of the Golgi apparatus widen somewhat as they extend away from the central area. Characteristically, at the tip of the cisternae, there are numerous vesicles. These appear to have arisen from certain of the cisternae (Figs. 2,5). These vesicles appear to have been released into the cytoplasmic system (Fig. 3). In addition, the Golgi apparatus seems to be associated with the endoplasmic reticulum which in turn may be continuous with the

Fig. 1.   Basidium of _Coprinus_ _lagopus_ showing intra-

cellular organization:  N, nucleus at meiotic

prophase; c, centriole in association with

the outer nuclear membrane; M, mitrochondria;

V, vacuole; ER, endoplasmic reticulum; R,

ribosomes; G, Golgi apparatus.  Note: the

endoplasmic reticulum (small arrows) connecting

the Golgi apparatus and the outer nuclear

membrane (large arrow).  The scale indicates

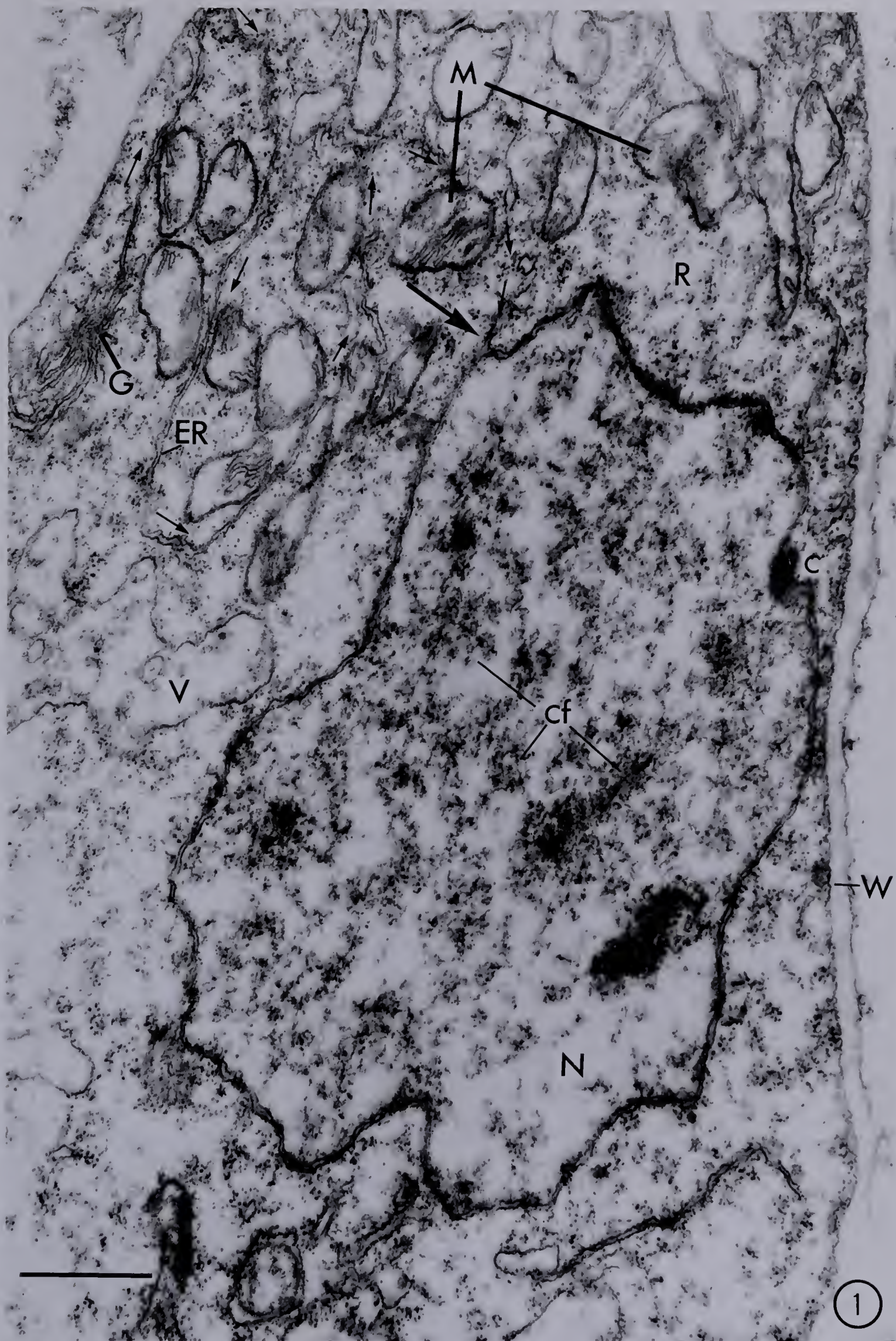1 micron, magnification approximately 22,000 X.

Fig. 2.    Golgi apparatus (G), as seen in a basidium,

in close association with the diploid nucleus

(N) at meiotic prophase.  Note that Golgi

vesicles arise from certain of the Golgi

cisternae.  The scale indicates 1 micron

magnification approximately 53,400 X.


Fig. 3.    Golgi apparatus (G), as seen in a basidium

in which the nucleus is at meiotic prophase.

Note Golgi vesciles (GV) around the Golgi

zone.  The scale indicates 1 micron, magni-
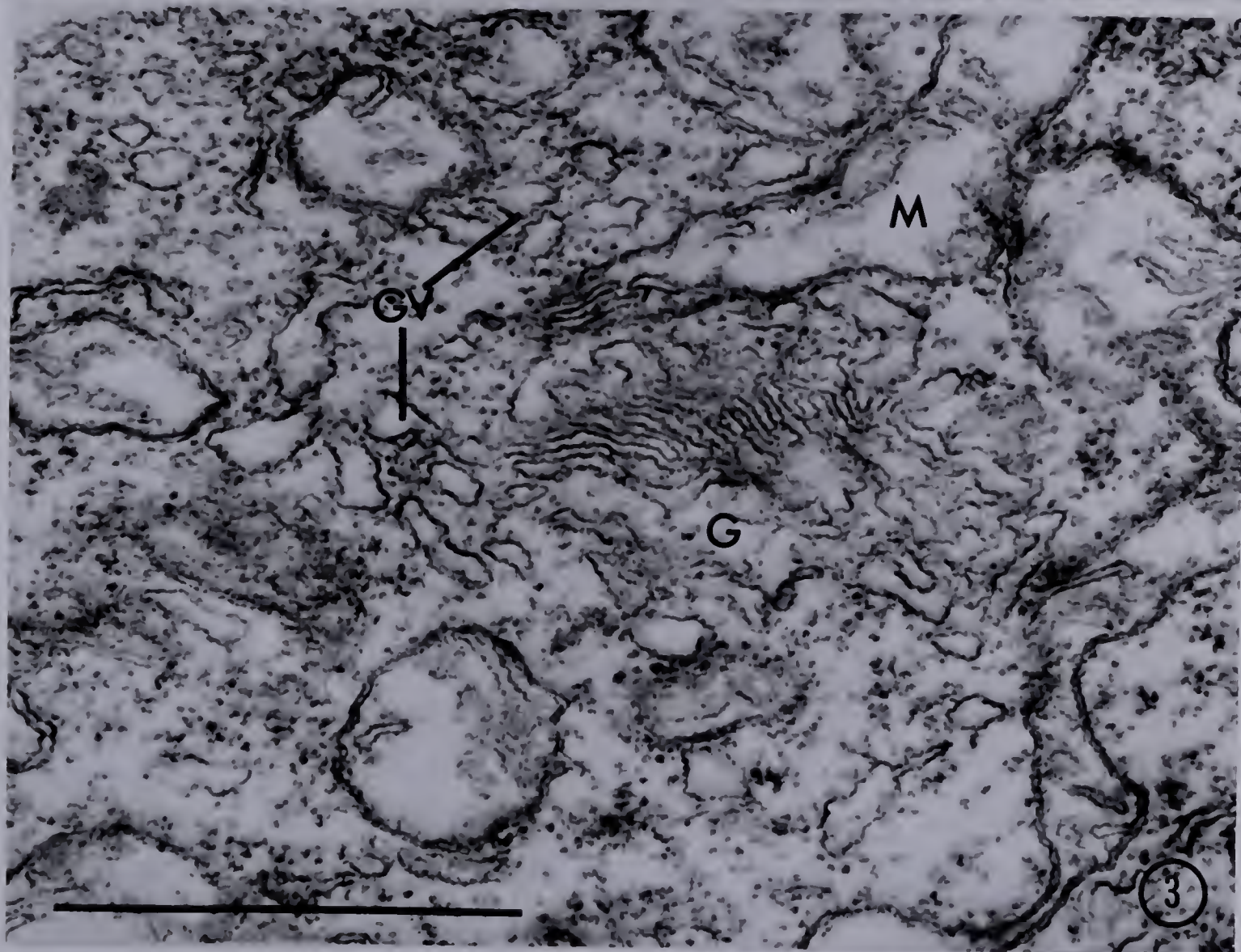
fication approximately 56,300 X.

Fig. 4.    Golgi apparatus (G), as seen in a basidium in which the nucleus is at meiotic prophase. Note that the endoplasmic reticulum (ER) is continuous with a Golgi cisterna.  Fig. 4 - Fig. 1 -- Fig. 5 are serial sections.  The scale indicates 1 micron, magnification approximately 60,000 X.
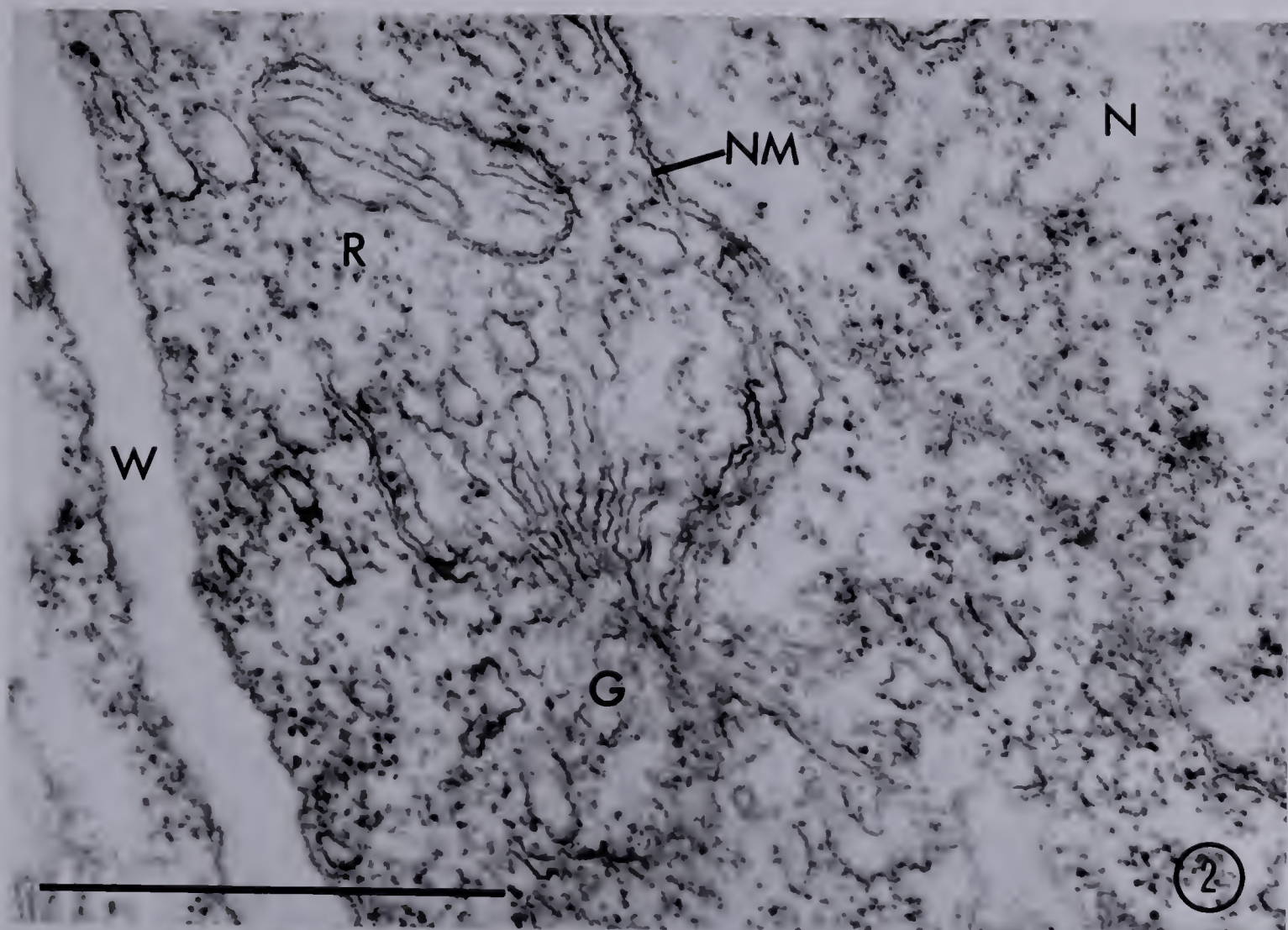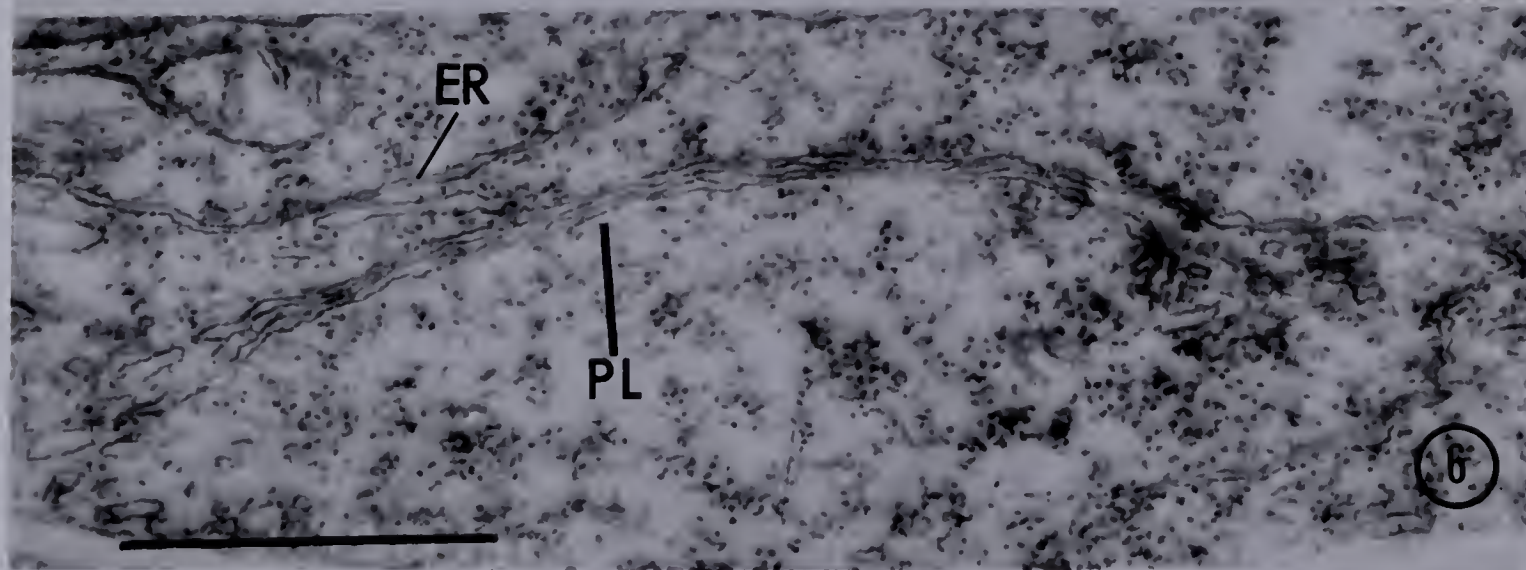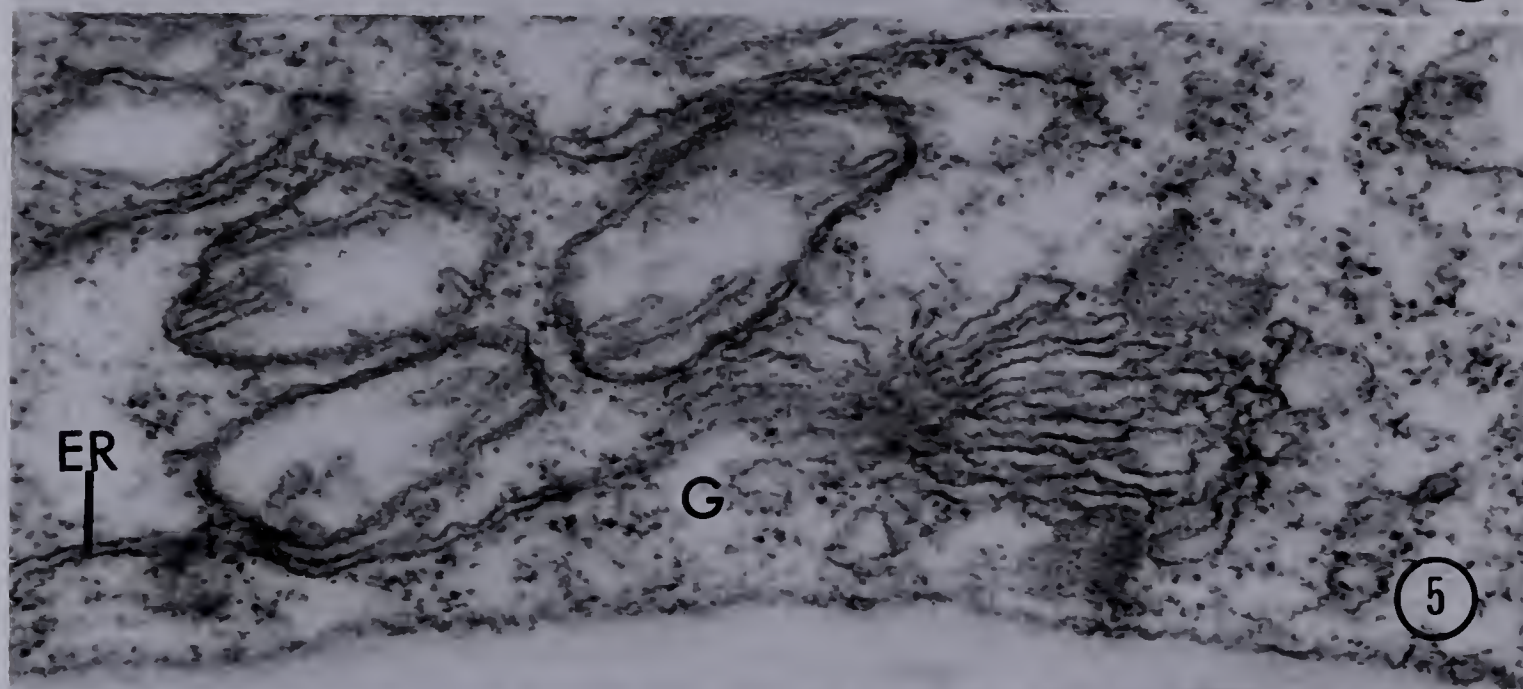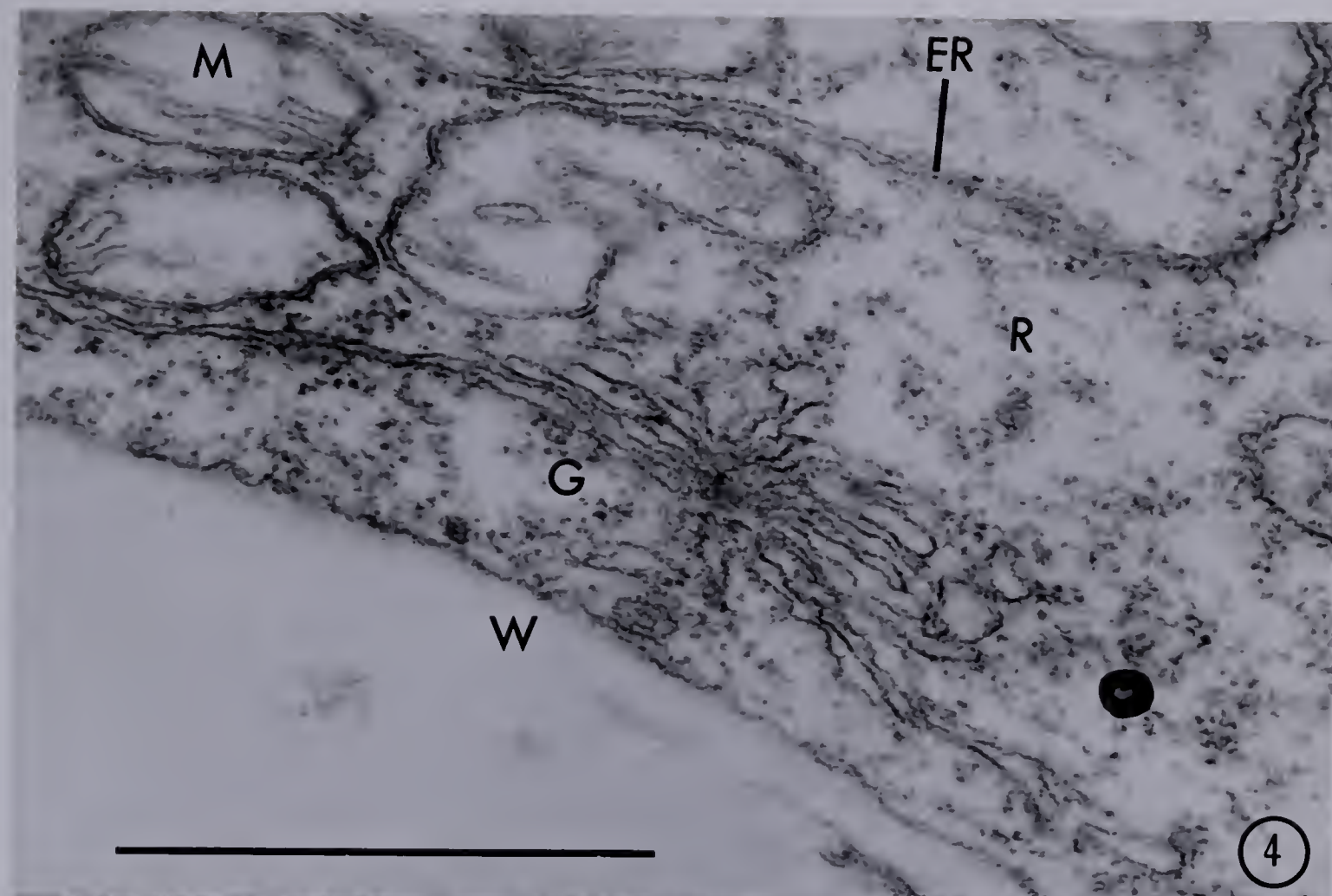
Fig. 5.    Golgi apparatus (G), as seen in a basidium in which the nucleus is at meiotic prophase. Note that Golgi cisternae radiate in all directions.  (A diagram of Golgi apparatus is show in Fig. 5a,.

Fig. 6.    Parallel lamellae (PL); note vesicles arising from ends of each cisterna.  The scale indicates 1 micron, magnification approximately 36,500 X.
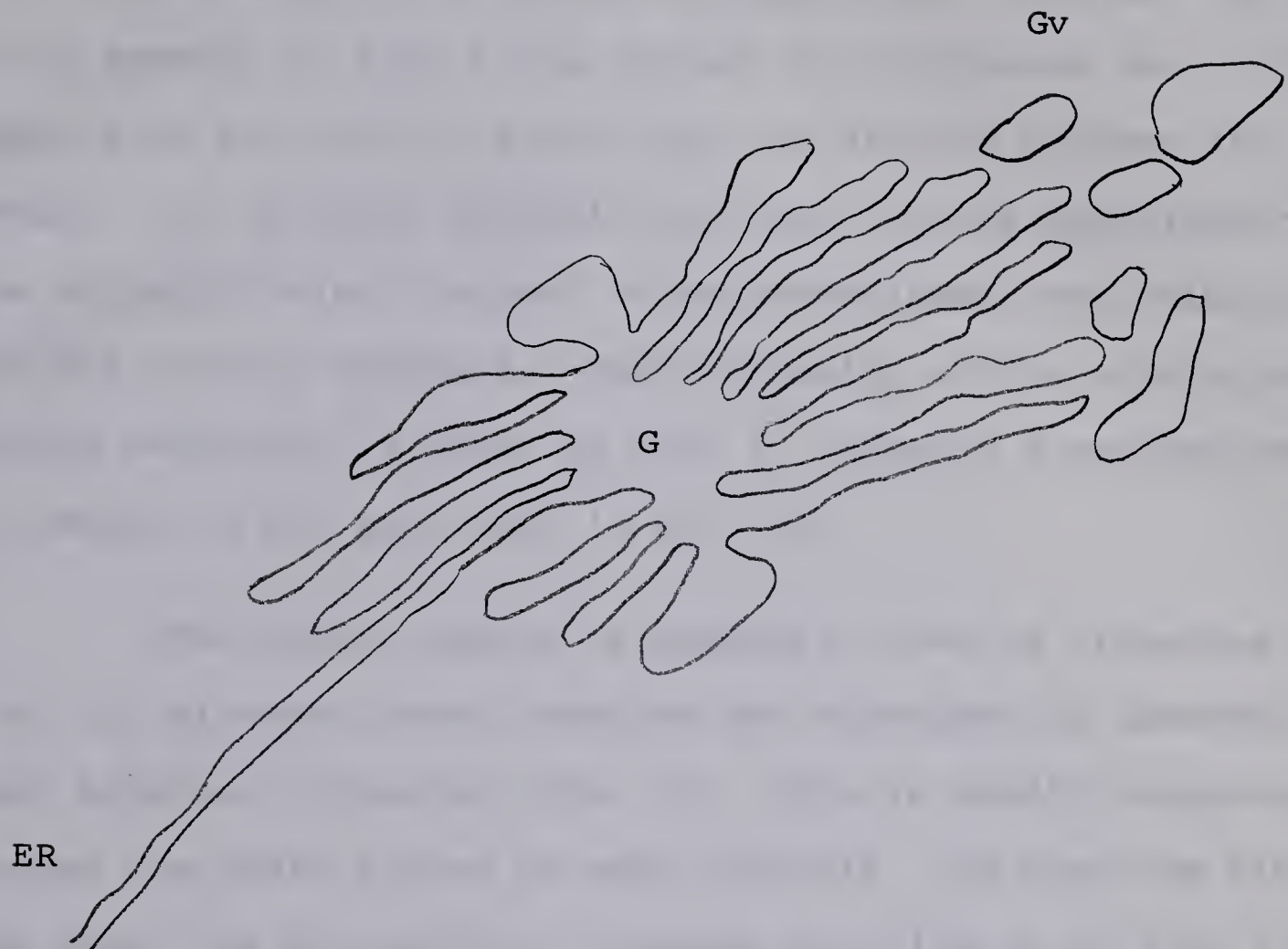
M
ER
R
G
W
4

ER
G
5

ER
PL
6

Fig. 5a. A composite diagrammatic illustra-
tion of the Golgi apparatus as shown in Fig.
5. Magnification approximately 100,000 X.

nuclear membrane (Fig. 1, large arrow).  In Fig. 1 is shown a
portion of a basidium, in which a Golgi complex is seen (at the
left) to be continuous with the endoplasmic reticulum.  The
latter appears to turn twice through the cytoplasm and to dis-
appear from the section plane near the diploid nucleus (Fig. 1,
arrows).  It is quite possible that the winding endoplasmic retic-
ulum actually joins the part of the endoplasmic reticulum continuous
with the nuclear membrane.  The continuity of the winding endo-
plasmic reticulum is shown in Fig. 4, (showing a section next in
the series to the one shown in Fig. 1).

The Golgi complex is generally free of ribosomes (Figs.
3, 4, 5), although these granules may sometimes be observed be-
tween adjacent cisternae (Fig. 2).  This is hardly surprising,
because the whole system is very flexible, and when the cisternae
flow apart the surrounding ribosomes may flow in to fill the gaps.

It can be seen that the complex found in the basidium,
in which the nucleus is at pachytene-diplotene stage, is relative-
ly small, being 1 μ long by 0.5 μ wide.  It has short lamellae
and has rather few marginal vesicles (Figs. 2, 5).  However, when
the nucleus of the basidium enters into metaphase I of meiosis,
the Golgi apparatus becomes greatly expanded.  As shown in Figs.
7, 8 and 9, it is then five to six times larger than that found
at pachytene.  The Golgi cisternae at this stage appear to be
irregularly blebbed to form numerous small vesicles (Fig. 7).
Nevertheless, the parallel cisternal arrangement is still
preserved (Fig. 7, arrow).

Fig. 7.  Golgi apparatus (G), as seen in a basidium
         in which the nucleus is at meiotic metaphase
         I.  Note Golgi vesicles (GV) exceptionally
         expanded.  The arrow marks the parallel Golgi
         cisternae.  The scale indicates 1 micron,
         magnification approximately 40,000 X.
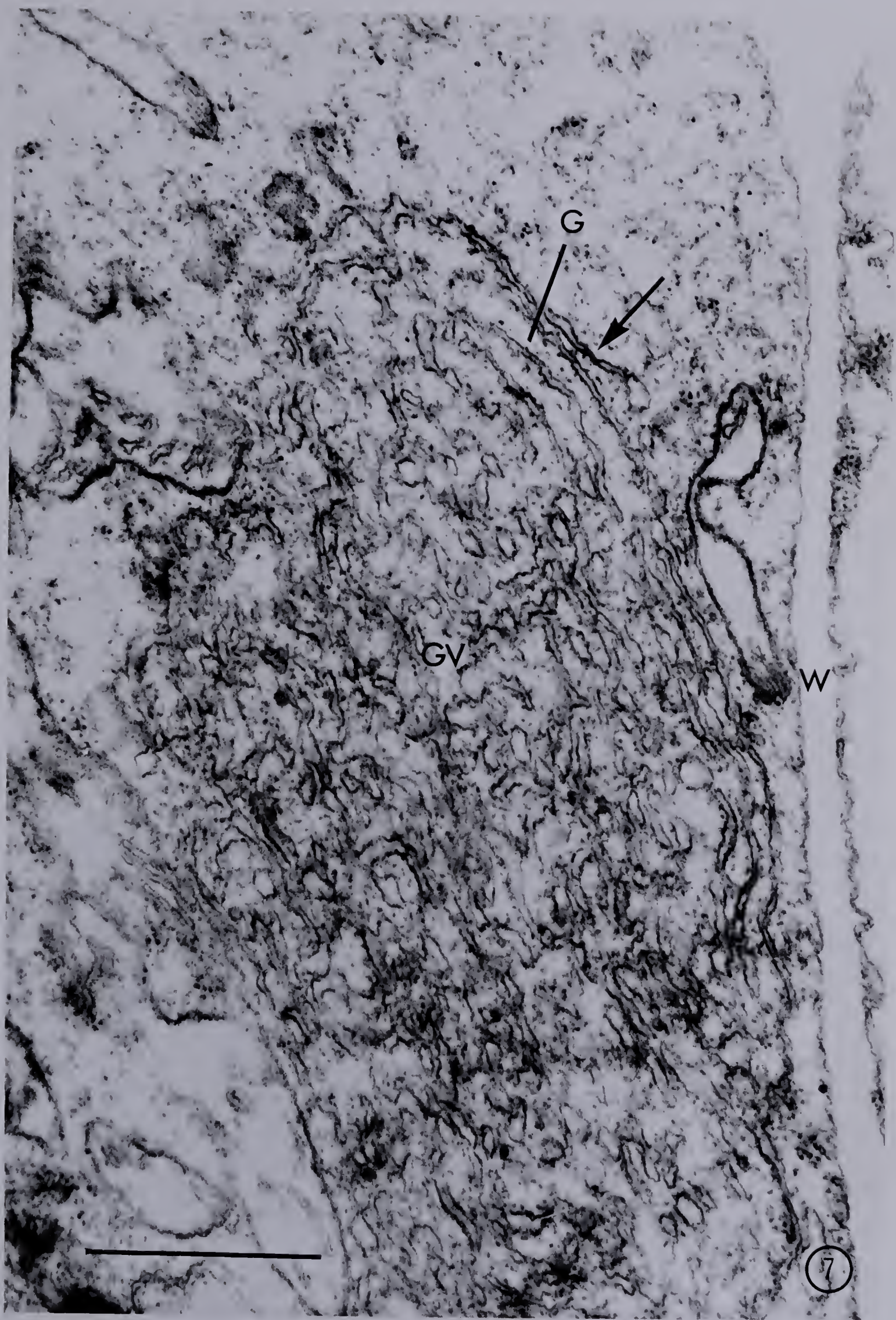
Fig. 8.   Basidium of <u>Coprinus</u> <u>lagopus</u>, in which the
          nucleus is at meiotic metaphase I, showing
          Golgi vesicles (G) in close association
          with the spindle zone (the spindle is cut
          obliquely).  The scale indicates 1 micron,
          magnification approximately 20,400 X.


Fig. 9.   The lower part of the basidium shown in Fig.
          8:  S, septum; G, Golgi apparatus; f, fat
          globules; R, ribosomes.  The scale indicates
          1 micron magnification, approximately 24,000 X.

8



9
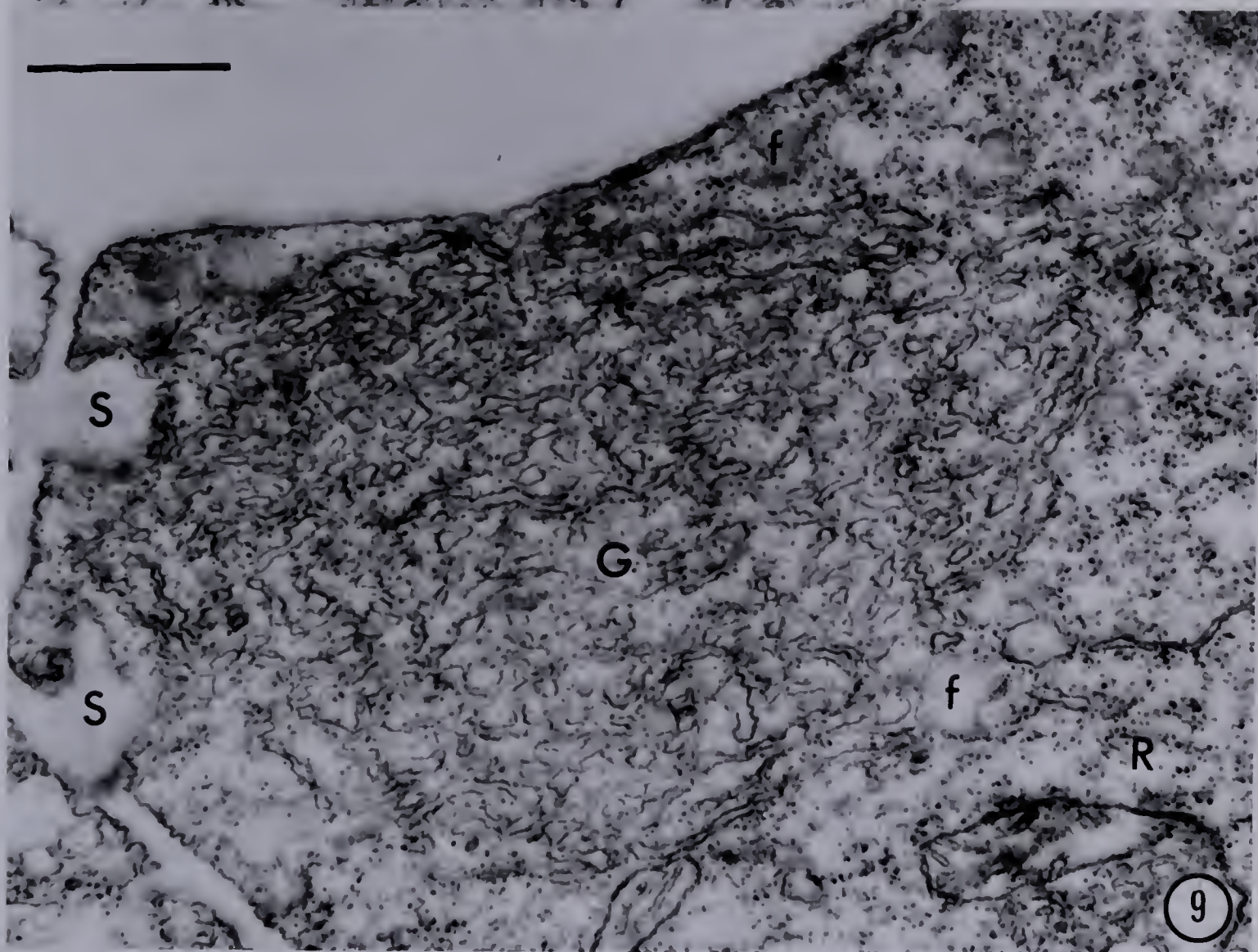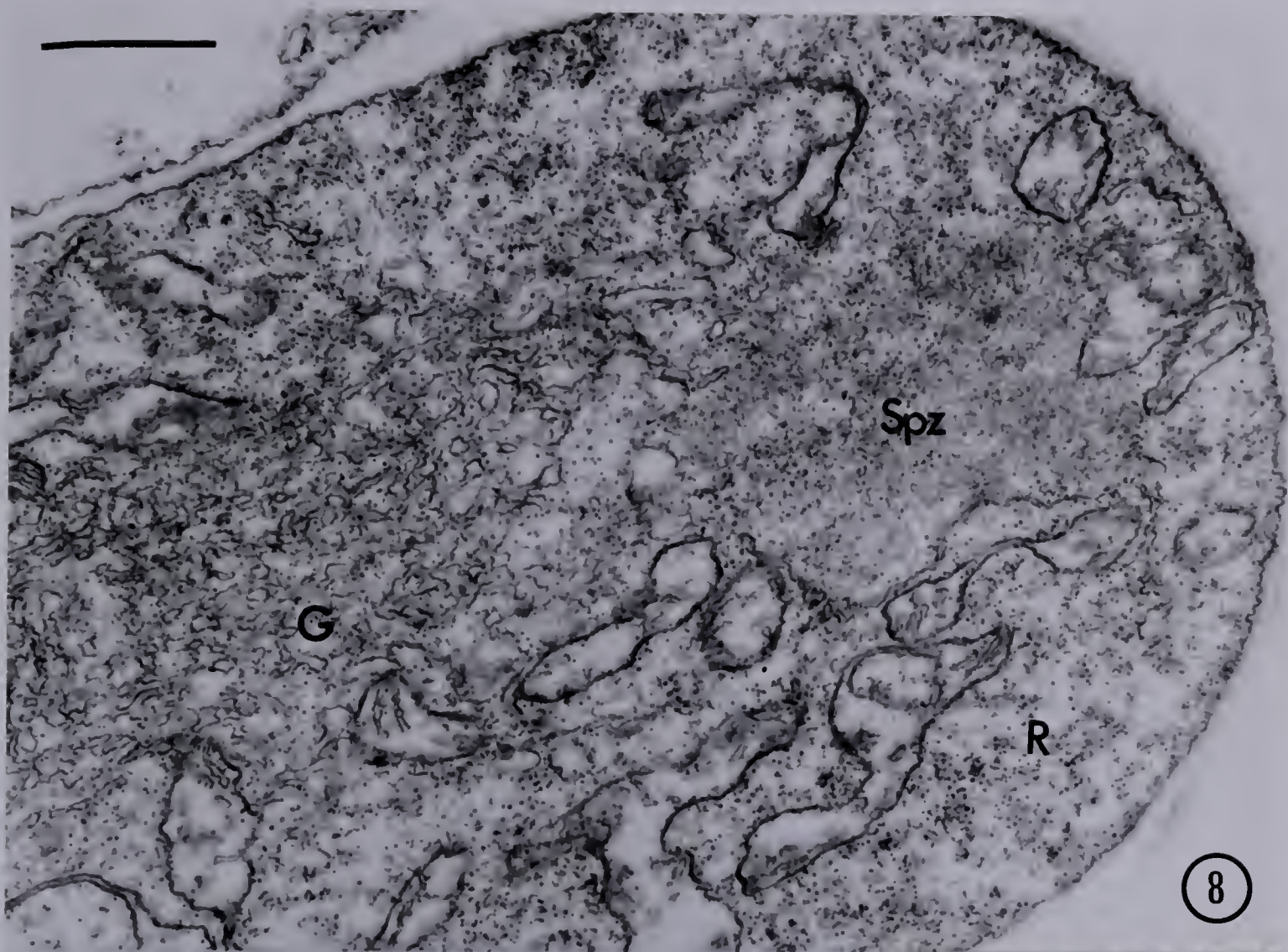
There are two such complexes in a basidium, one on top
and the other at the bottom (Fig. 8, 9; two named illustrations
represent one basidium). The former is near the metaphase spindle
zone. As seen in Fig. 58, the Golgi vesicles are closely assoc-
iated with the spindle zone near a centriole.

The sudden change of the Golgi activity is noteworthy.
Its occurrence exactly at premetaphase or metaphase of meiosis
preceding two consecutive nuclear divisions strongly suggests
that the Golgi complex may be producing membrane precursors
for the organization of new nuclear membranes. Such membrane
synthesis may, indeed, constitute the major function of the
Golgi apparatus.

2. Ribosomes

Ribosomes are abundant in the basidium of Coprinus
lagopus. They are suspended in the cytoplasmic ground sub-
stance (Figs. 10, 11, 12, 13). Their distribution, however, is
by no means random. In the basidium, they appear to be strung
together in short chains of varying numbers of components (Figs.
10, 11, arrows). These aggregates may represent polyribosomes,
which consist of a varying number of single ribosomes attached
to a single-stranded messenger RNA, and are the sites of in vivo
protein synthesis or of polypeptide chain synthesis (Wettstein,
et al., 1963; Staehelin, et al., 1964; Rich et al., 1964). In
the chains shown in these figures, all the ribosomes appear to
be equally spaced. This conclusion is in good agreement with

Fig. 10.  A portion of a basidium of <u>Coprinus</u> <u>lagopus</u>
         showing free ribosomes (R).  The arrow points
         to linear aggregates, the polyribosomes.
         Magnification approximately 93,000 X.


Fig. 11.  A portion of a basidium showing free ribo-
         somes (R) and the smooth-surfaced endoplasmic
         reticulum (ER).  The arrow points to a linear
         aggregate of ribosomes, the polyribosome.
         Magnification approximately 97,000 X.

NM

N

R

0.5 μ

10

ER

R

Vc

0.5 μ

11
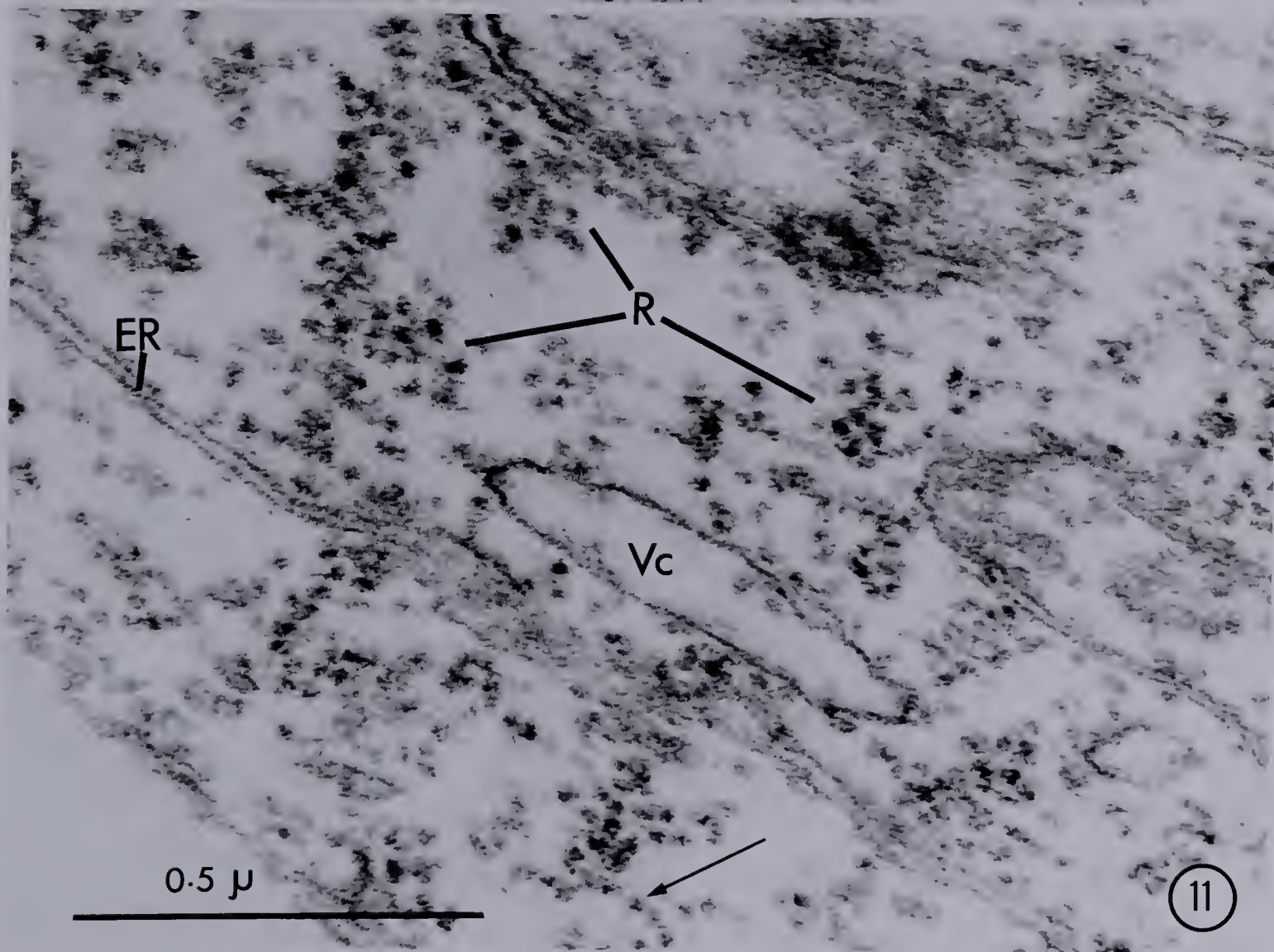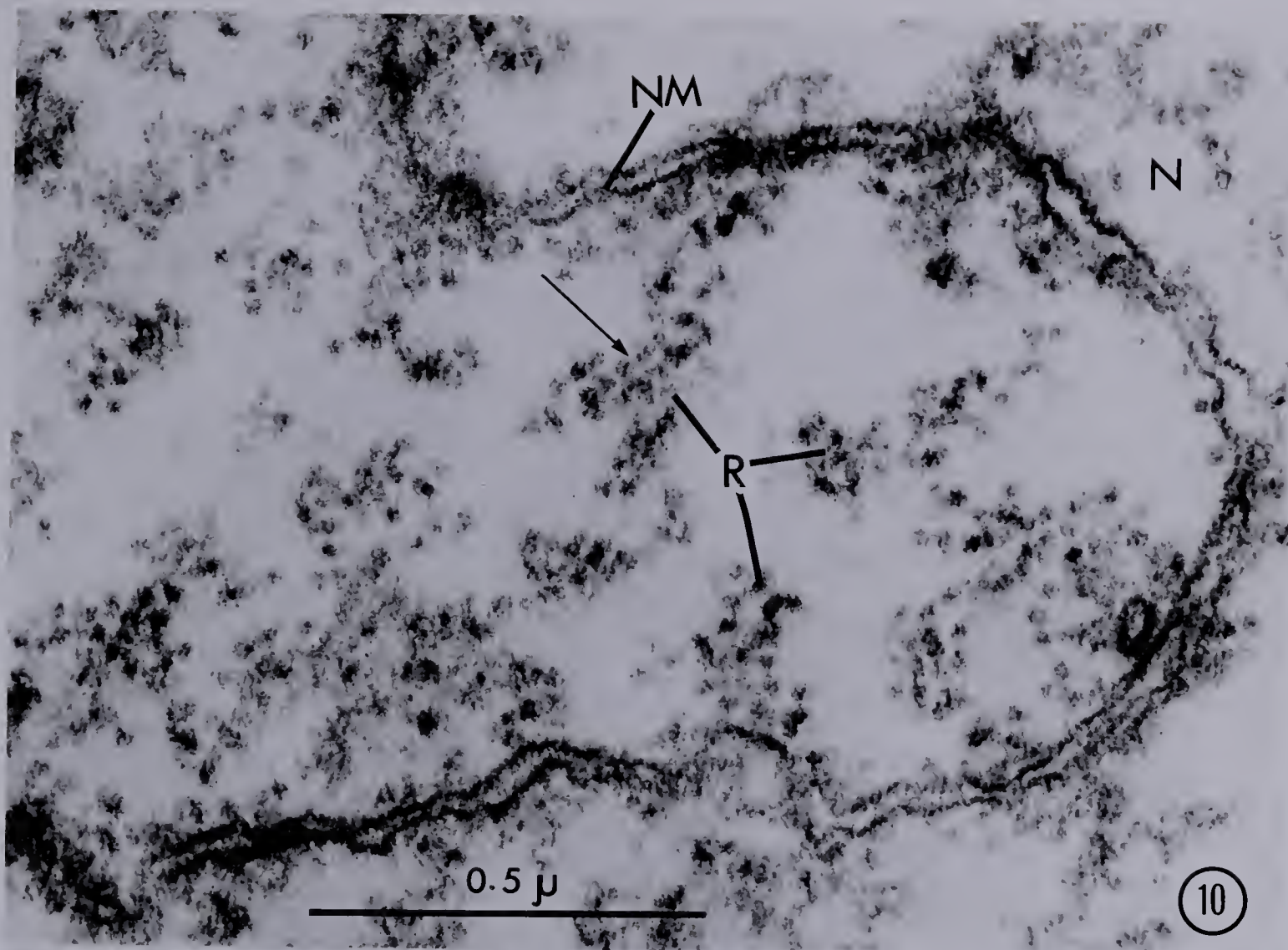
the current concept that, in a polyribosome chain, two adjacent ribosomes are about 90 nucleotide units apart (Staehelin et al., 1964). Similar polyribosome chains have been demonstrated in intact reticulocytes (Riefkind, Luzzatto and Paul, 1964) and in rat liver cells (Dass et al., 1965) by means of electron microscopy.

The ribosomes measure about 150 - 300 Å in diameter. They probably represent particles which exhibit a sedimentation coefficient of 80 S during ultracentrifugation, as demonstrated by Huxley and Zubay (1960) and by Taylor and Storck (1964). In high resolution electron micrographs, ribosomes appear to include fine electron-dense fibrils of about 20 - 30 Å in thickness (Fig. 11). The electron-dense fibrils may represent ribonucleoprotein strands.

3. The Membrane System

The membrane system of cells of the fungus Coprinus lagopus resembles that of other organisms. It consists of the plasma membrane, the endomembrane, the tonoplast, and other membranes. In the present context, only the plasma membrane and that of the endoplasmic reticulum are described.

The plasma membrane is the outermost layer of the protoplast closely pressed against the cell wall. It is made up of a single unit membrane about 70 Å thick, which, in a high resolution electron micrograph, may be seen to consist

Fig. 12.   A portion of a basidium of <u>Coprinus</u> <u>lagopus</u>

showing; R, ribosomes; ER, endoplasmic retic-

ulum; N, nucleus.   The arrow marks the sur-

face view of the endoplasmic reticulum.   The

scale indicates 1 micron, magnification approx-

imately 44,000 X.


Fig. 13.   A portion of a basidium (bottom), near a

sterile cell (above), of <u>Coprinus</u> <u>lagopus</u>

showing: PM, plasma membrane which consists

of two electron-dense layers separated by a

clear space; R, ribosomes; M, mitochondria;

C, centriole.   The scale indicates 1 micron,

magnification approximately 53,300 X.

N

R

ER

⑫

PM

W

M

C

R

⑬
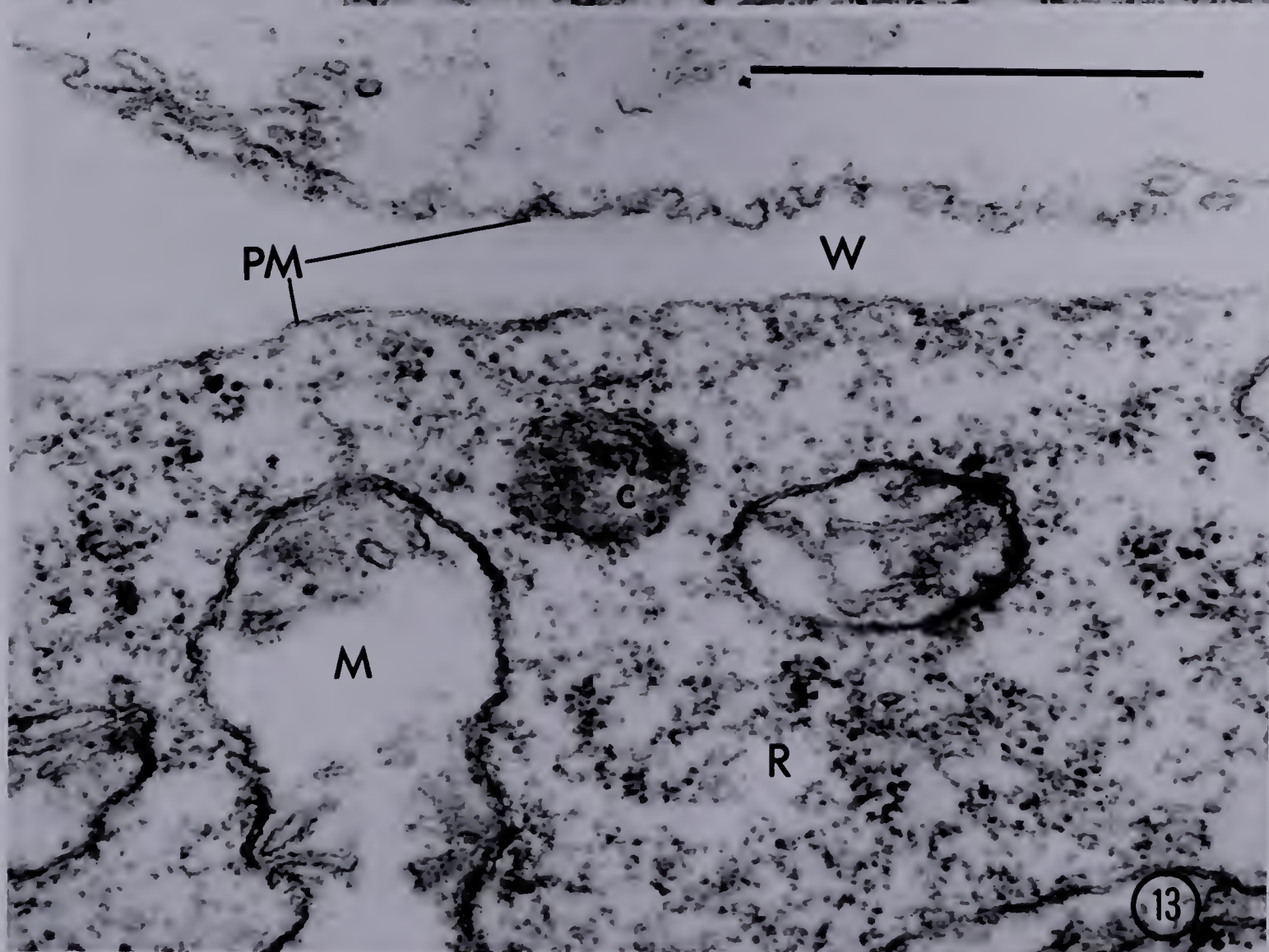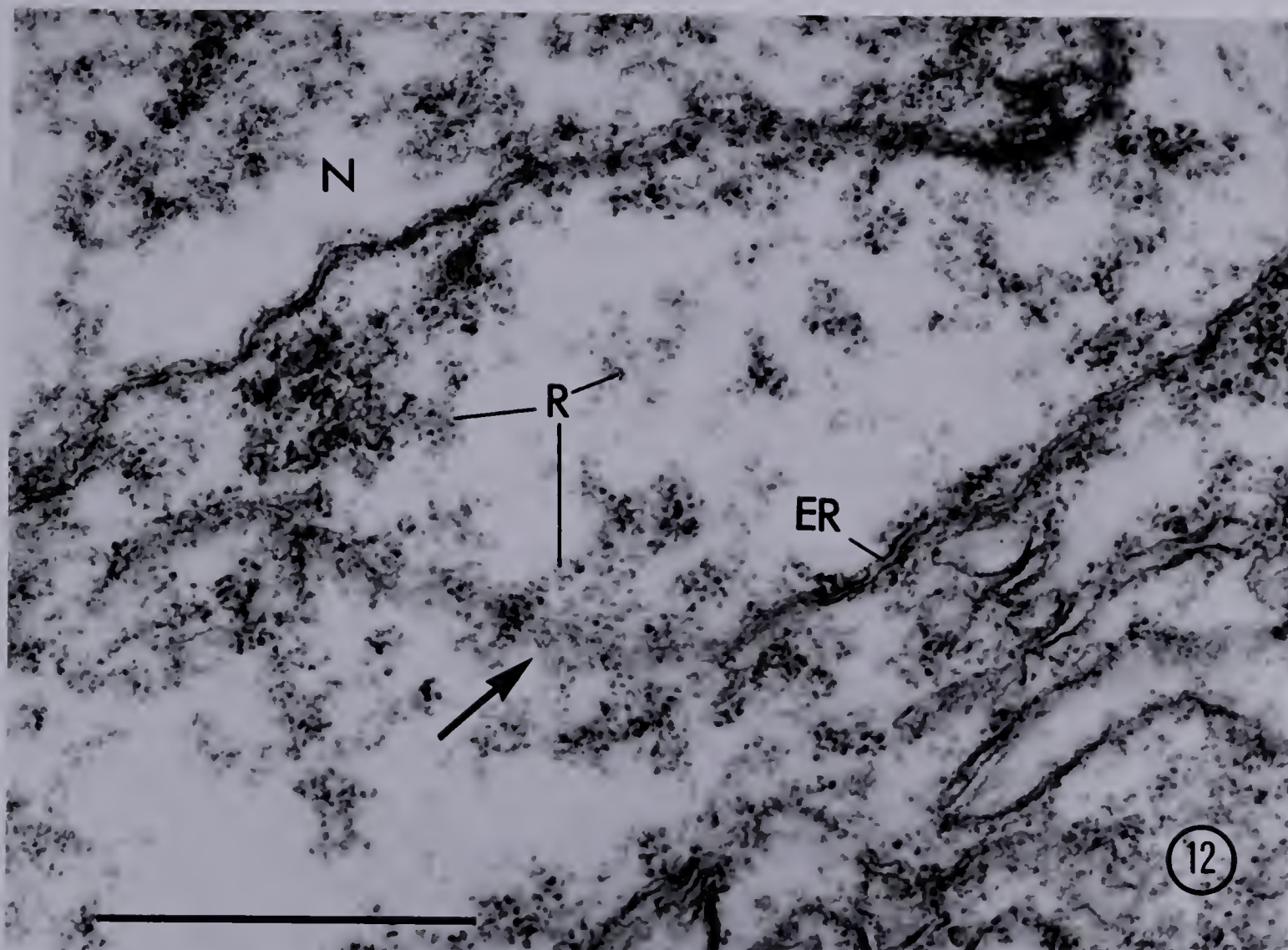
of two electron-dense layers (Fig. 13) approximately 30 $\overset{o}{A}$ thick,
separated by a clear space. Membranes have been seen to be
invaginated into the cytoplasm in some places.

The membrane of the endoplasmic reticulum of Coprinus
cells is itself essentially free of ribosomes (Figs. 11, 12).
However, this endomembrane may appear to be crowded with ribosomes,
as a result of random distribution of the latter in the cytoplasmic
matrix, this gives one the impression that the membrane of the
endoplasmic reticulum of Coprinus is beset with ribosomes in a
manner comparable to the rough-surfaced ergostoplasm of animal
cells. However, careful examination reveals that the ribosomal
density on the membrane as shown in a surface view (Fig. 12,
large arrow) is not any greater than that of the nearby aggregates.
In Fig. 11, moreover, membranes of the endoplasmic reticulum are
seen to be free of ribosomes. This is also true for the membrane
of a vesicle. Thus, the endoplasmic reticulum of the fungus
Coprinus may be considered to be a smooth-surfaced membrane
system.

In thin sections, the endoplasmic reticulum appears
to comprise two unit membranes separated by a clear space of
variable width. It may appear as long narrow cisterna or as an
ovoid vesicle (Fig. 11). It is highly branched, as can be seen
in Figs. 14 and 16 which may imply that the endoplasmic reticulum
forms a continuous communication for the cell system, as pointed
out by Porter (1961). Morphologically, it resembles the nuclear

Fig. 14.  Mycelium from the pseudoparenchymatous

tissue of _Coprinus lagopus_ showing the

endoplasmic reticulum.  Arrows mark branches

of the membranes.  The scale indicates 1

micron, magnification approximately 31,200 X.


Fig. 15.  A portion of a sterile cell of the hymenium

showing vacuole (V) continuous with the

endoplasmic reticulum (arrows).  The scale

indicates 1 micron, magnification approximately

18,000 X.

ER

14

V

15
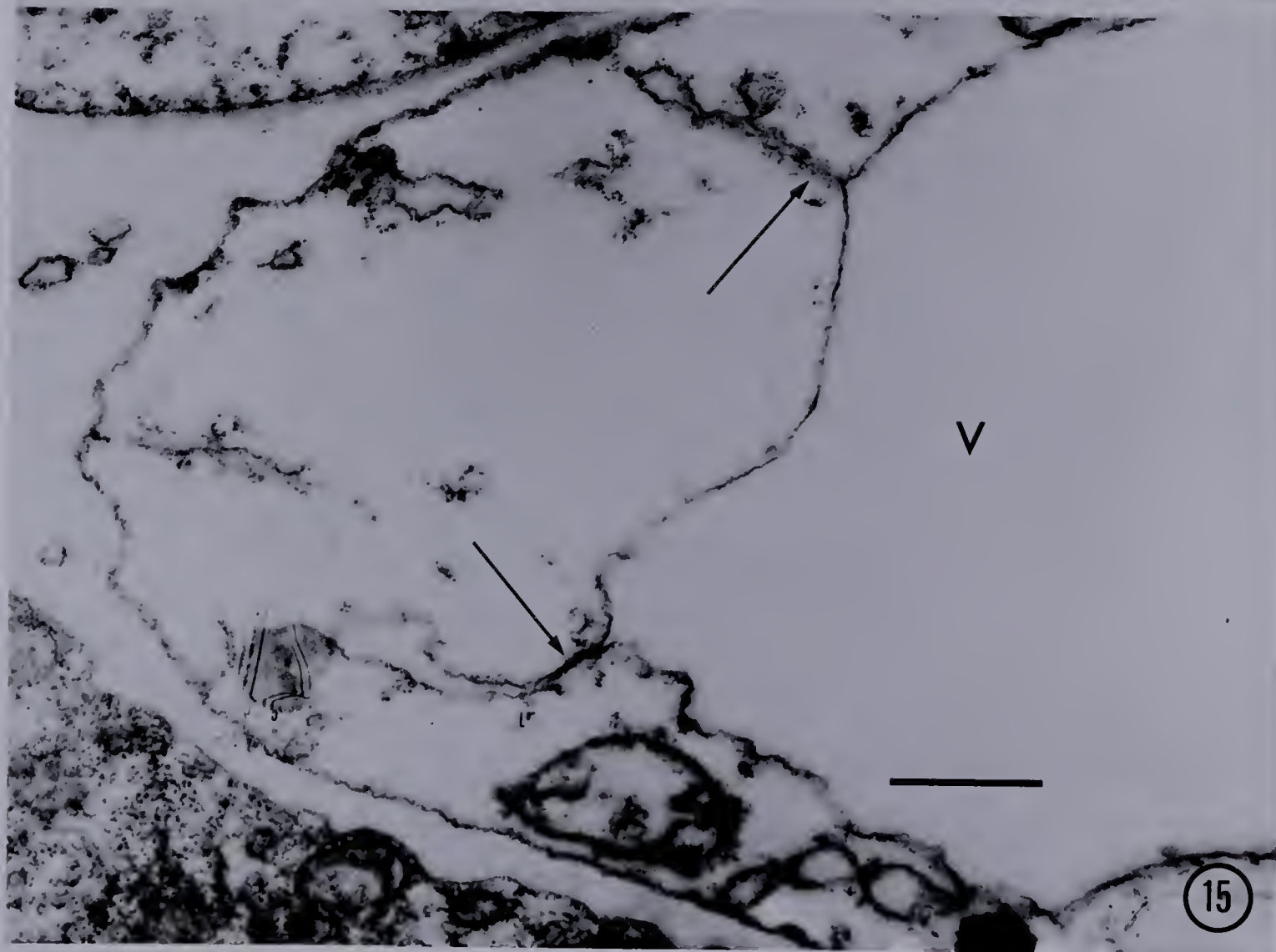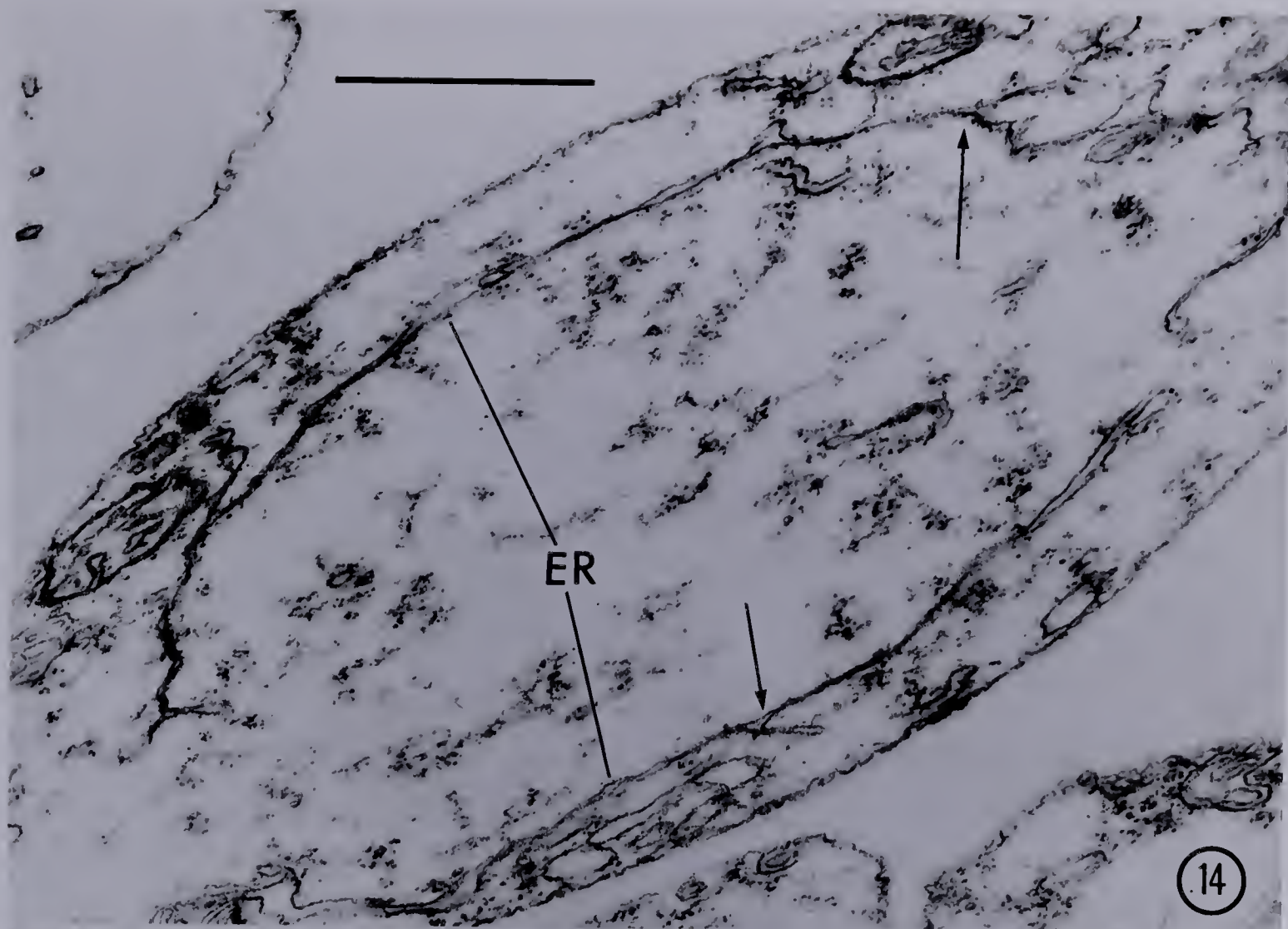
membrane and the Golgi membrane. Evidence now accumulating indi-
cates that all these three membranes are interconnected (Dalton,
1961; Wells, 1965; Moore and McAlear, 1962a; McAlear and Edwards,
1959).

4. Vacuoles

Vacuoles are generally associated with the aging of
a cell. They may be important in the maintainence of cell turg-
idity, at least as far as plant cells are concerned. In a young
basidium, before the fusion of two compatible nuclei, vacuoles
are absent. As the basidium develops, vacuoles are observed
as early as the pachytene stage. These then increase in size
or in number as the basidium matures. Growth of vacuoles can
be observed by means of light microscopy, and can be demonstrated
by means of electron microscopy (Figs. 15, 17). In Fig. 15 is
shown a fully mature vacuole in a cystidium at the time when
spores are being produced.

As shown in Figs. 17 and 18, a vacuole is enveloped
by a single unit membrane (the tonoplast). Inside the membrane,
there are granules of unknown nature and origin (Fig. 18). These
granules disappear when the vacuoles mature (Fig. 15). Vacuoles
may be highly lobed; these appear to have several processes like
the pseudopods of amoeba (Figs. 15, 17). This may be attribut-
able to the plasticity of the tonoplast, or may be related to
the way the vacuole is formed (see following).

Fig. 16.   Basidium of _Coprinus lagopus_, at the spore-
           producing stage, showing a highly branched
           endoplasmic reticulum (ER) which is probably
           in the process of forming a vacuole.  The
           scale indicates 1 micron, magnification
           approximately 32,000 X.


Fig. 17.   Basidium of _Coprinus lagopus_, showing a
           vacuole (V) which probably arises from the
           expansion of the branched endoplasmic
           reticulum.  Magnification approximately
           32,000 X.

ER

(16)



V

(17)
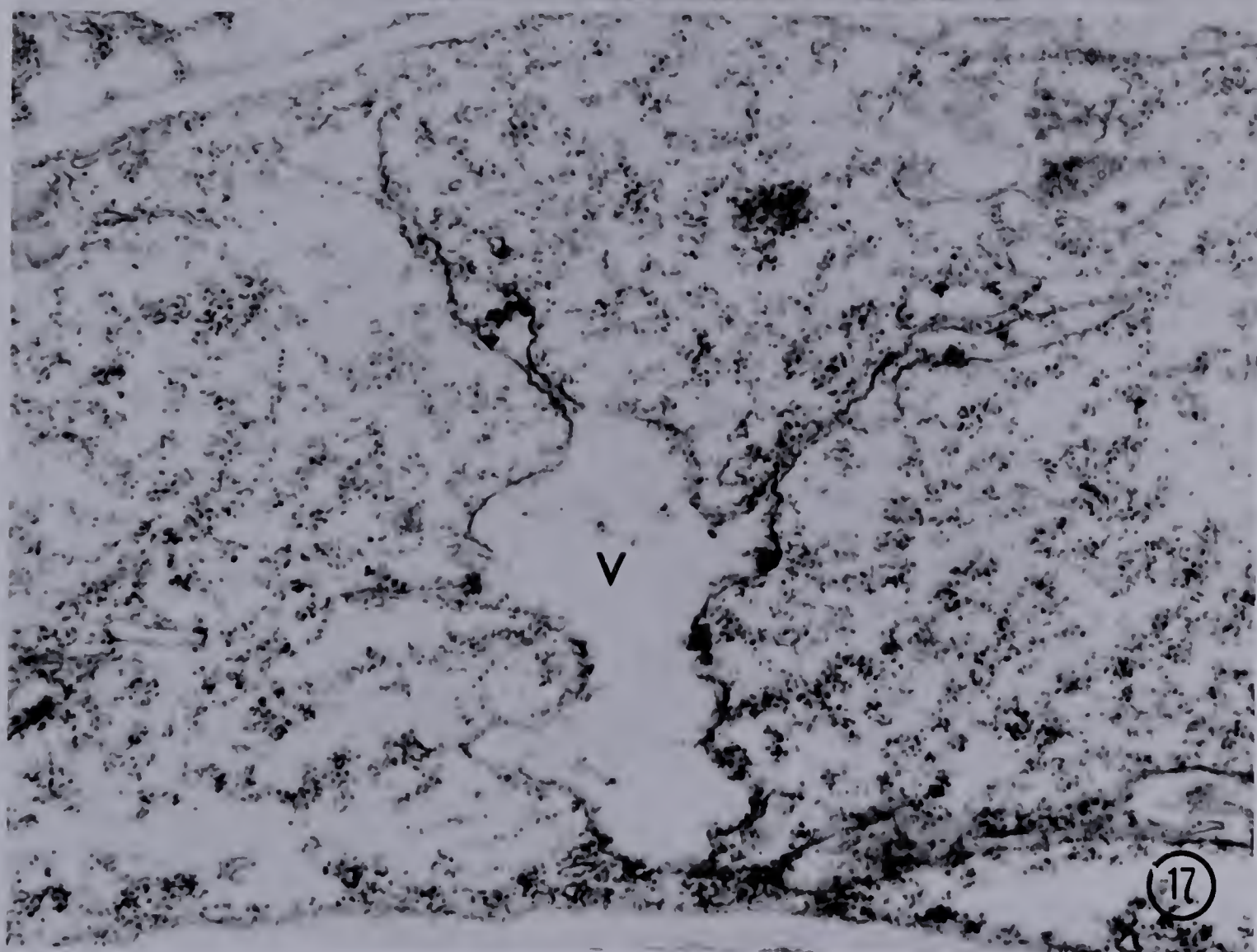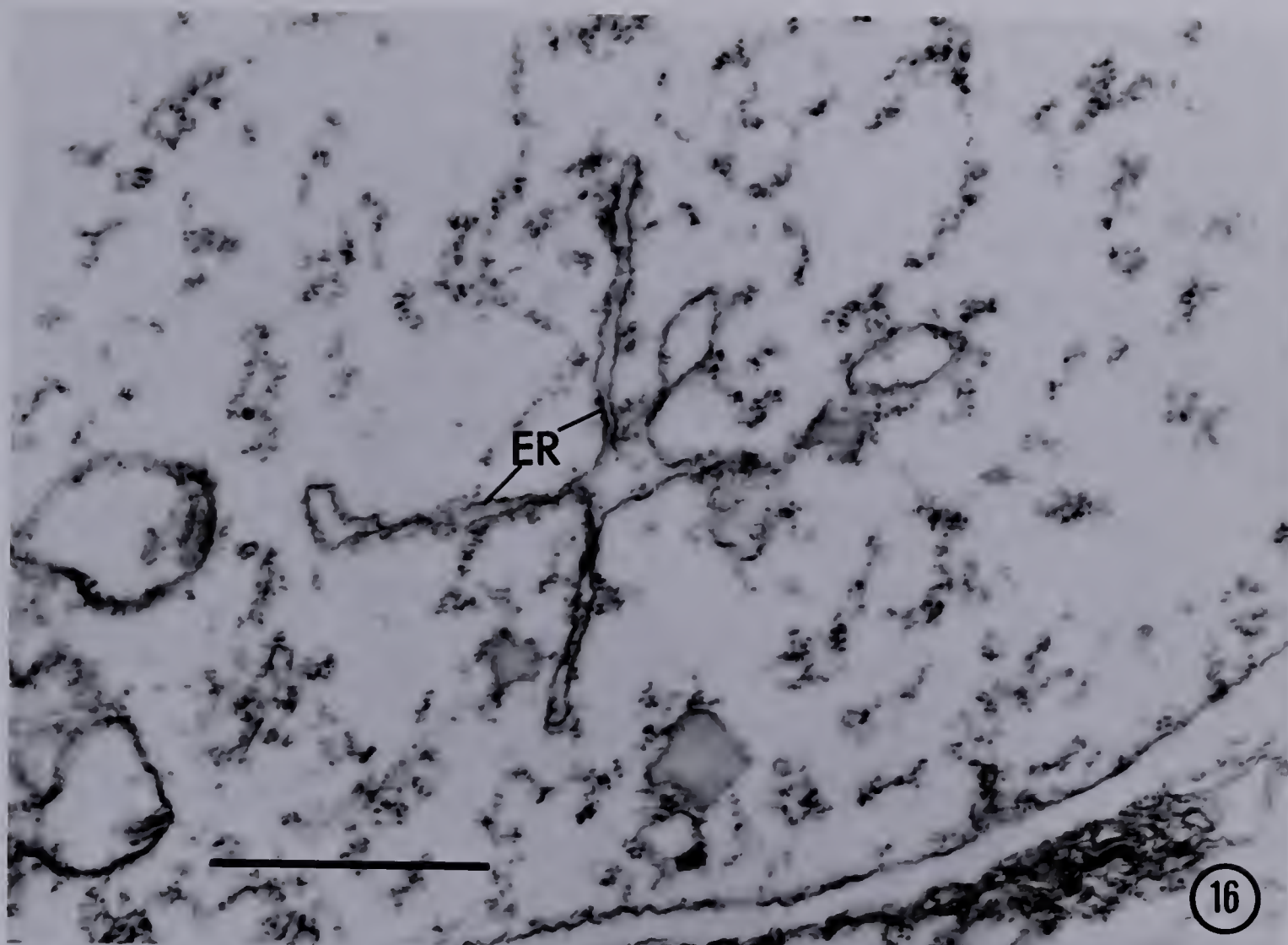
It is interesting to note (in Fig. 18) that the endo-
plasmic reticulum is swollen at the left end (v). Also in this
figure is shown a small vacuole connected to a large one by the
endoplasmic reticulum (arrow at the left). These observations
suggest that a vacuole is formed by the process of separation of
two membrane layers of the endoplasmic reticulum. The branched
endoplasmic reticulum shown in Fig. 16 may be expanding to form
a vacuole. This mode of vacuolar ontogeny may account for the
following facts: (1) the vacuoles have only a single unit mem-
brane and in this respect are different from all other cellular
organelles except the lysosomes; and (2) they are formed when the
cells grow old, although there has been no prevacuolar body. It
follows that the growth of vacuoles in a developing basidium is
probably accomplished by continuing expansion of the endoplasmic
reticulum. This view is strengthened by the observation that
there is little endoplasmic reticulum in the highly vacuolated
cystidia (Fig. 15). Also in Fig. 15, it is shown that the vacuole
is continuous with the endoplasmic reticulum (arrows).

5. Lomasomes

Lomasomes are characterized as vesicular aggregates
located outside the protoplast and delimited by the invagination
of the plasma membrane (Fig. 19). These vesicular aggregates
were found in the mycelium (Fig. 28, 29) and in the basidium
(Fig. 19). Lomasomes were first reported and named by Moore and
McAlear (1961a) in the mycelium of several species of fungi.
Similar structures were actually described earlier as "rings"

Fig. 18.    Basidium of Coprinus lagopus showing: N,

nucleus; Nu, nucleolus; ER, endoplasmic

reticulum; R, ribosomes; V, vacuoles.  The

arrow marks the ER connecting between two

vacuoles.  Magnification approximately

27,000 X.


Fig. 19.    Lomasome (Lo) as seen outside the plasma

membrane of a basidium of Coprinus lagopus.

Magnification approximately 40,000 X.


Fig. 20.    Lomasome (Lo), as seen inside the basidium

in which meiosis is complete; N, nucleus;

W, cell wall.  Magnification approximately

40,000 X.


Fig. 21.    Lomasome (Lo) as seen outside the plasma

membrane of a cystidium of Coprinus lagopus;

N, nucleus; PM, plasma membrane; W. cell wall.

Magnification approximately 49,000 X.
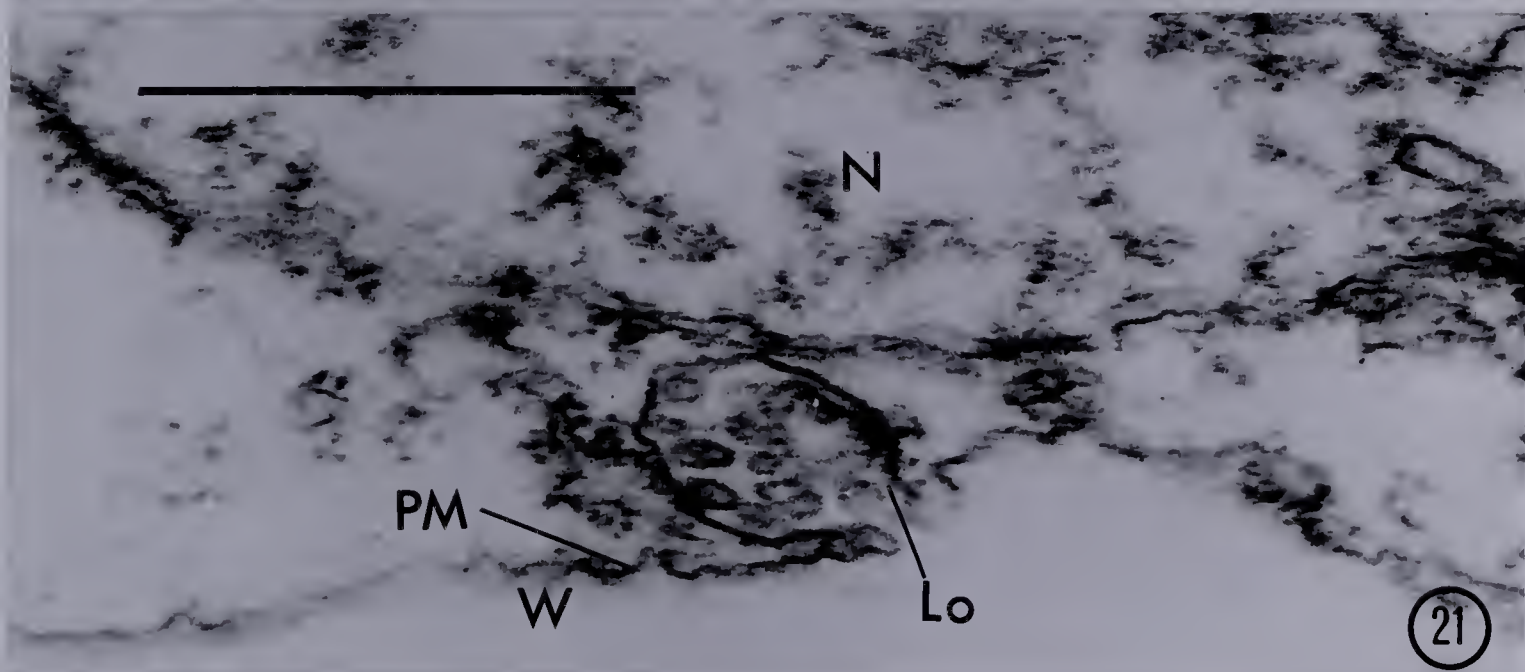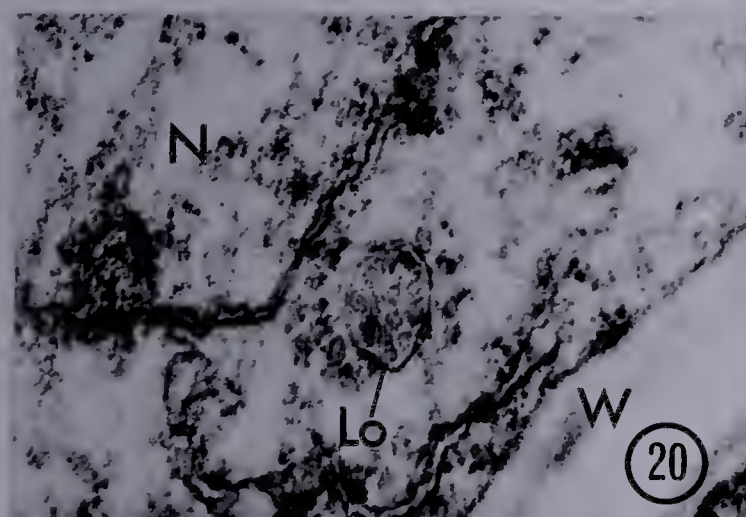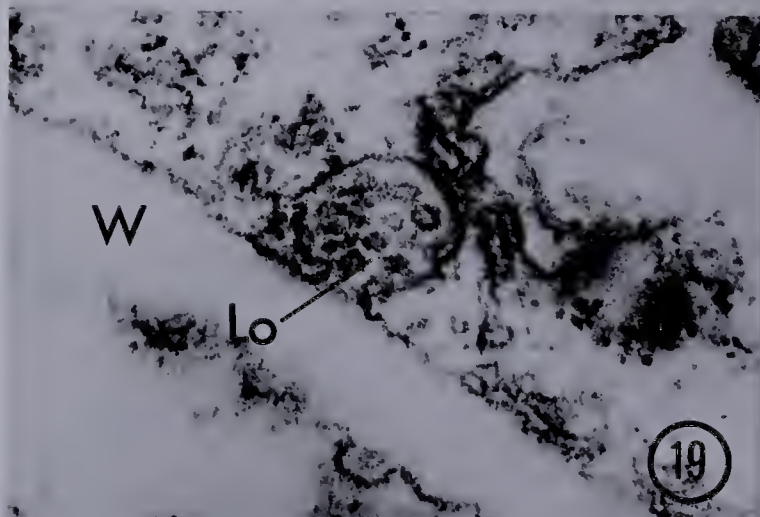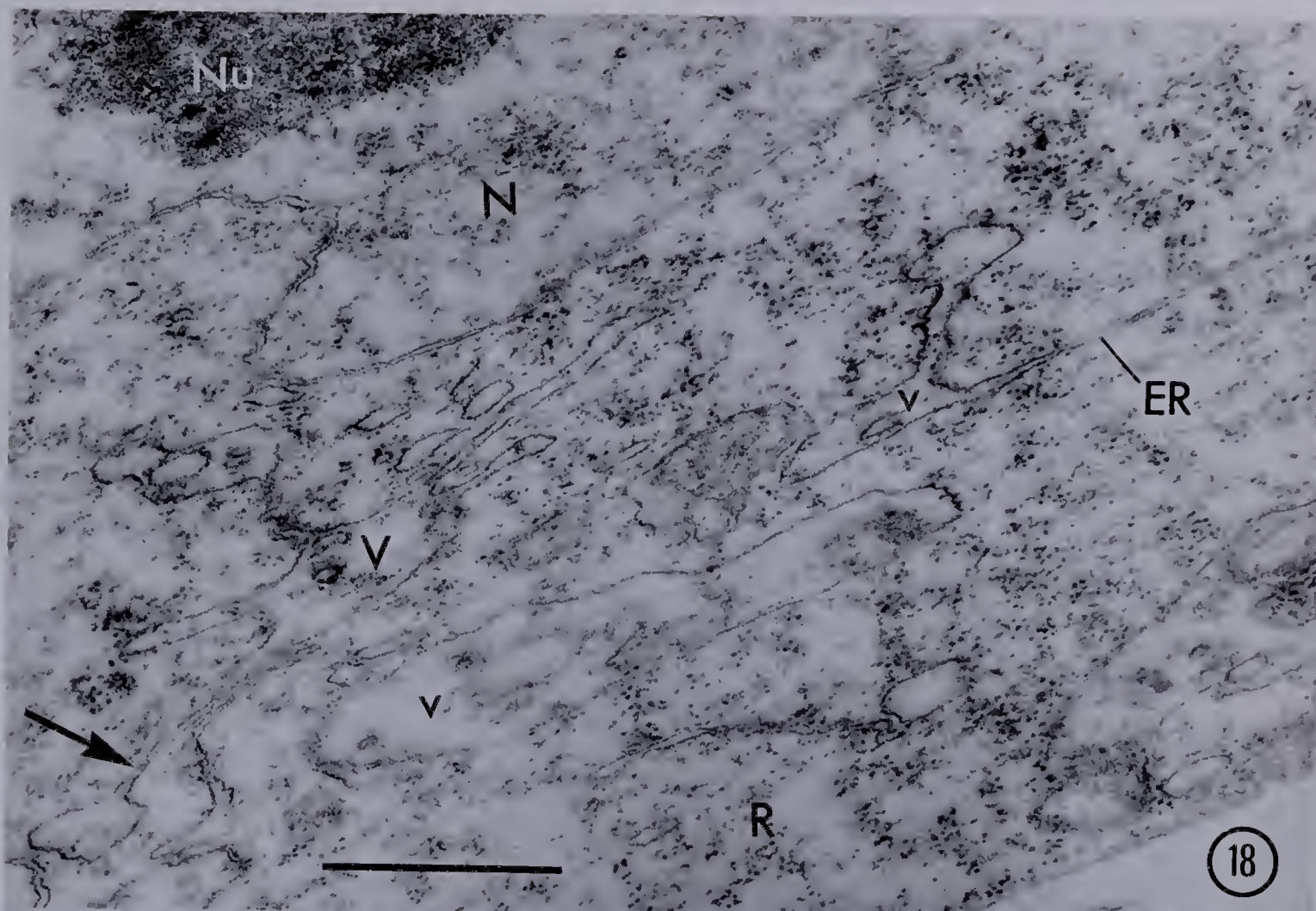
by Girbardt (1958), in <u>Polystictus versicolor</u>.  In the present
comparative studies, lomasomes were rarely observed in the early
developmental stages of the basidium, but were observed abundantly
in a basidium in which meiosis had been completed (Fig. 19).
These observations suggest tentatively that lomasomes are assoc-
iated with aging of the cell.

Structures resembling lomasomes, except that they are
enveloped by a single unit membrane, were also observed frequently
within a basidium in an advanced stage of development (Fig. 20).
As Moore and McAlear (1961) noted, these structures may eventually
migrate to, and fuse with, the plasma membrane (Fig. 21).

### 6. Mitochondria

The mitochondria of <u>Coprinus lagopus</u> resemble those of
other organisms.  They are enveloped by two layers of osmiophilic
membrane separated by a clear space.  The inner osmiophilic mem-
brane is invaginated into the central body to form numerous cristae,
which may be arranged any way in a mitochondrion (Fig. 22).  The
cristae may take the form of plates or tubules (Fig. 22).  In
the matrix of a mitochondrion, there are small particles about
the same size and shape as ribosomes (Fig. 22, arrows).  These
mitochondrial particles appear to be associated most frequently
with the inner osmiophilic membrane as well as with the cristae.

In a basidium of <u>Coprinus lagopus</u>, mitochondria are
very numerous in the apical half of the cell.  Such a polarity
of distribution in a single cell is striking.

Fig. 22. Basidium of <u>Coprinus</u> <u>lagopus</u> showing mit-
ochondria (M). Note that the cristae may
be platelets or tubules. Arrows mark the
mitrochondrial particles, similar in size
and shape to the ribosomes. Magnification
approximately 59,300 X.

f  R

M

22
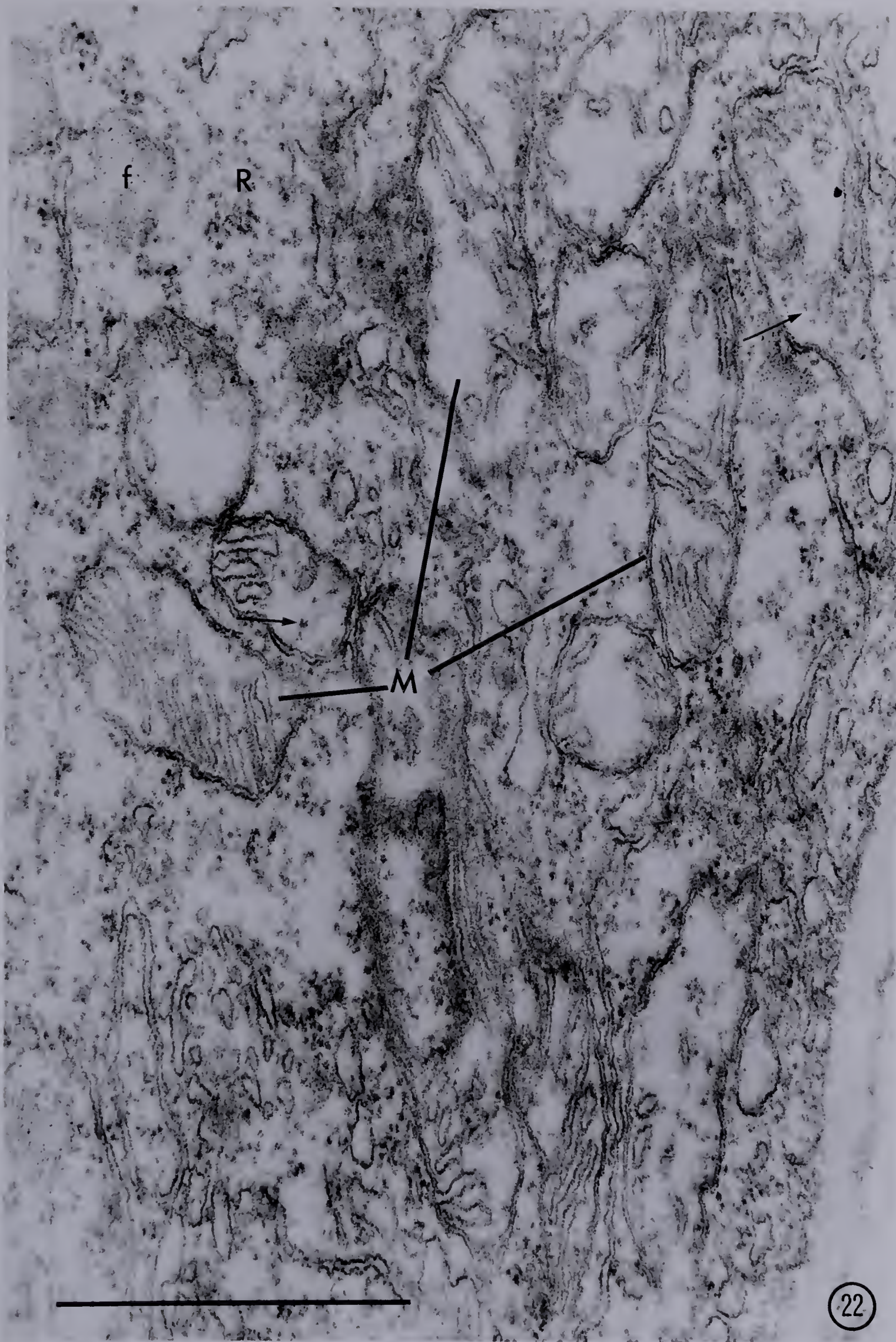
Fig. 23.  Mitrochondria of the basidium of Coprinus
         lagopus.  Note that the size of these is
         almost as long as the diploid nucleus.
         Magnification approximately 24,500 X.


Fig. 24.  Mitochondria (M) of the basidium of Coprinus
         lagopus showing invagination of the membrane
         (arrows).  Magnification approximately 62,000
         X.


Fig. 25.  Mitochondria (M) showing invagination of
         mitochondrial membrane (arrows).  Magnifica-
         tion approximately 20,000 X.
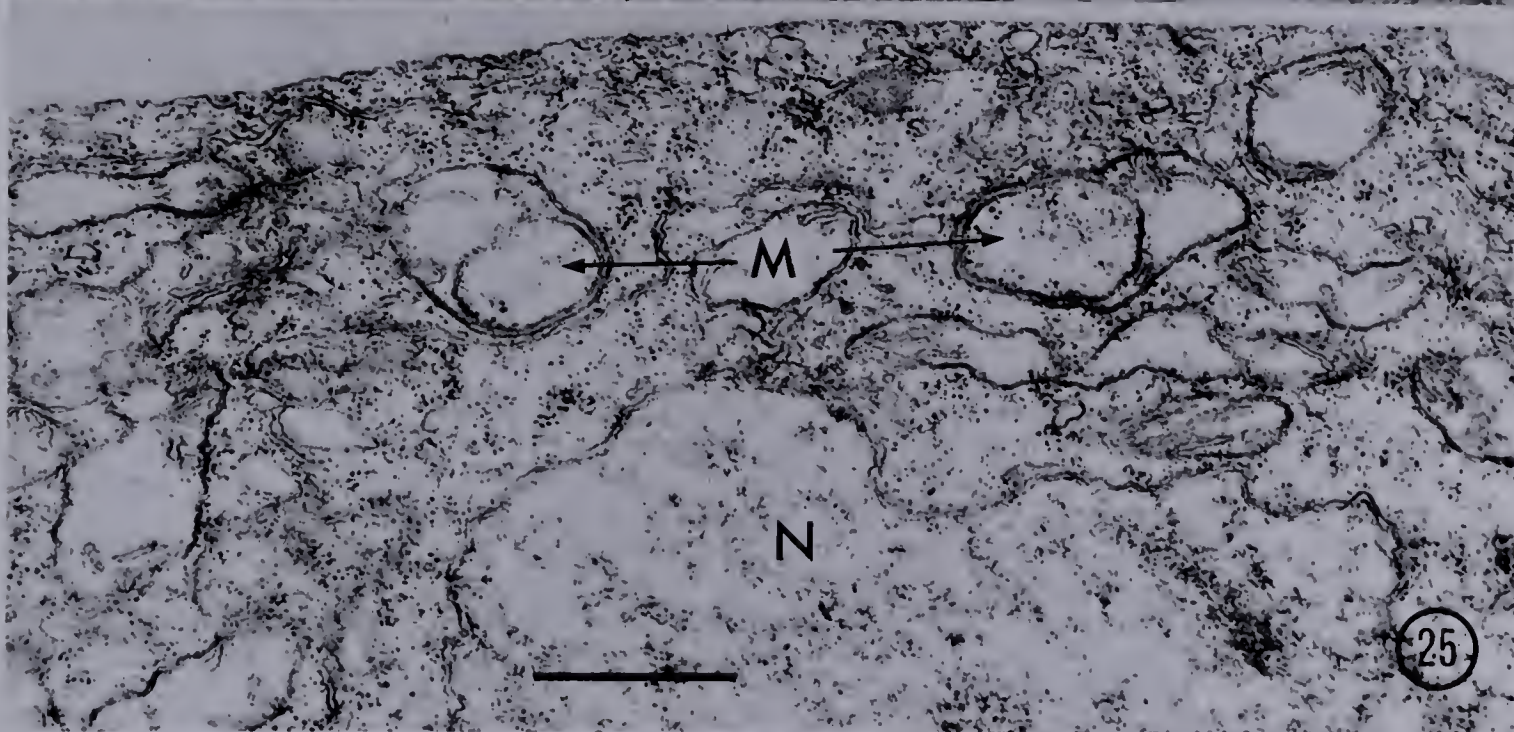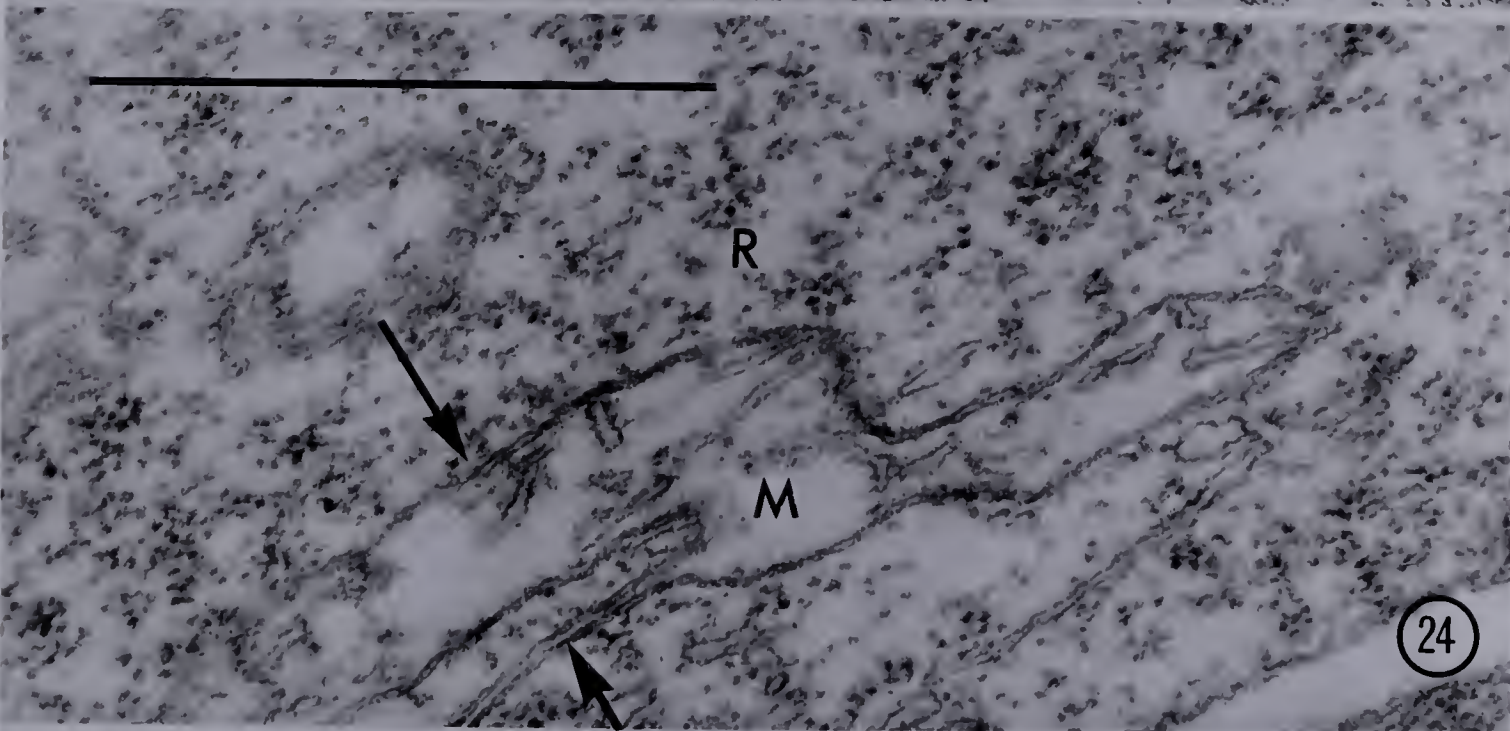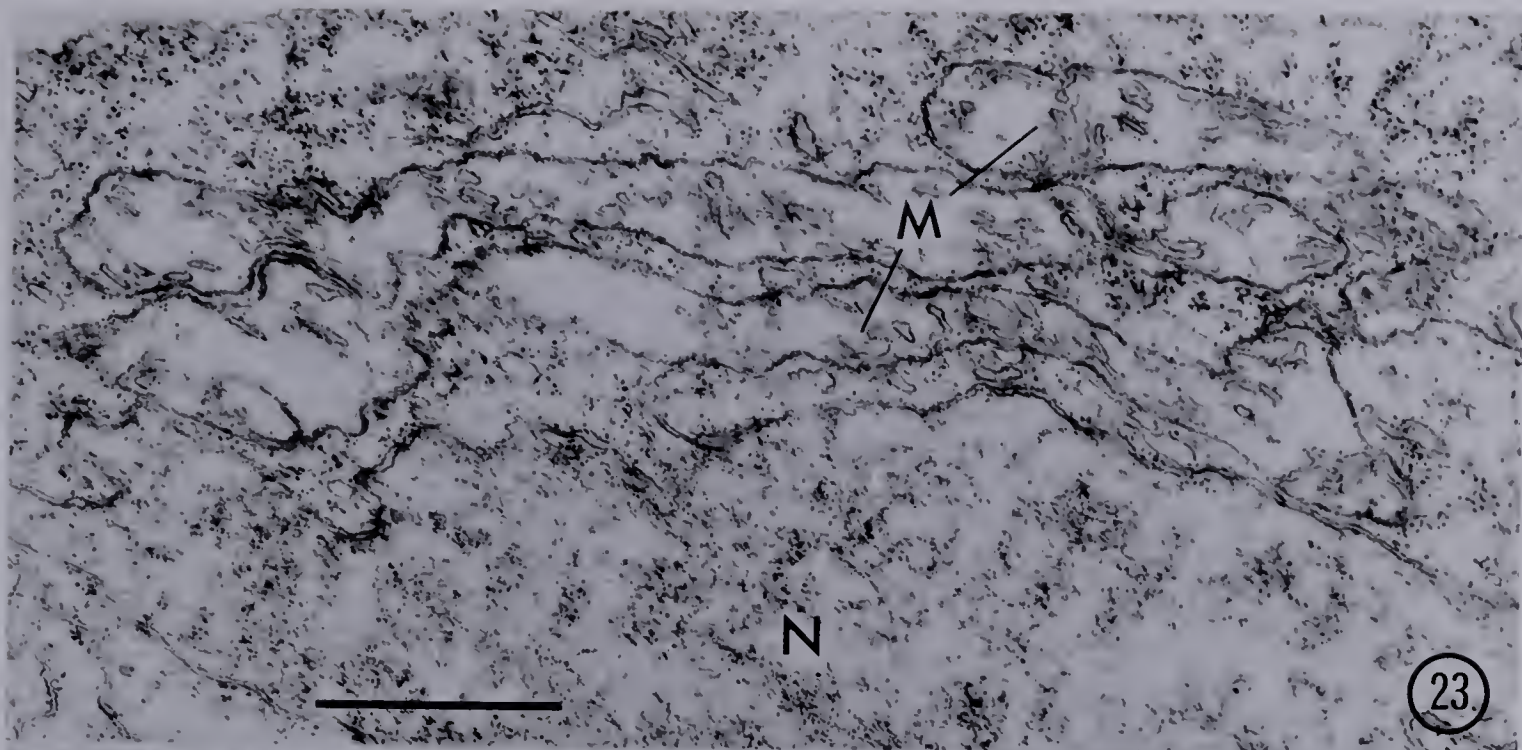
The mitochondria vary greatly in size and in shape. In thin sections, they may range from 0.5 to 7.0 µ in length (Figs. 22, 23). A mitochondrion may be constricted at the middle as though it were pinching into two. It may form amoeboid pseudo-pods or branches (Fig. 24). If a section were made through a pseudopod as indicated in Fig. 24 (arrows→ ←) the pseudopod would appear as an "atypical form" of a mitochondrion such as has been described by Stephens and Bils (1965). In Fig. 25 are shown two such "atypical forms" (arrows). They may not be atypical; rather their form may represent a quite general situation resulting from the plasticity of the mitochondrial membrane.

7. Septal Pore

The septal pore of Coprinus lagopus is a special feature of basidiomycetes. It is made up of a septal swelling which is like a plug with a canal in its center connecting two adjacent cells. The structure of the pore can be visualized from a cross section (Fig. 27) and a longitudinal section through the central pore region (Figs. 26, 27). The plasma membrane is continuous between two "cells". On each side of the plug, there is a bell-shaped septal cap composed of three membrane layers (Figs. 26, 27). This cap has been called the "parenthosome" by Moore and McAlear (1962c). It appears to be rigid and discontinuous (Figs. 26, 27). A similar kind of septal cap was reported by Berliner and Duff (1965) for Armillaria mellea, and by Giesy and Day (1965) for Coprinus lagopus. It is thus suggested that the rigid septal

Fig. 26.   Septal pore of mycelium of <u>Coprinus lagopus</u>.

S, septal swelling; P, septal pore; Scp,

septal cap; R, ribosomes; ER, endoplasmic

reticulum.  The arrow marks the ribosomes

transferring through septal-cap perforations,

Magnification approximately 32,000 X.


Fig. 27.   Septal pore of mycelium of <u>Coprinus lagopus</u>

showing a longitudinal section (upper) and

a transverse section (below).  W, cell wall;

S, septal swelling; P, septal pore; Scp,

septal cap.  Arrows mark the perforation

of septal cap.  Magnification approximately

35,300 X.

26



27
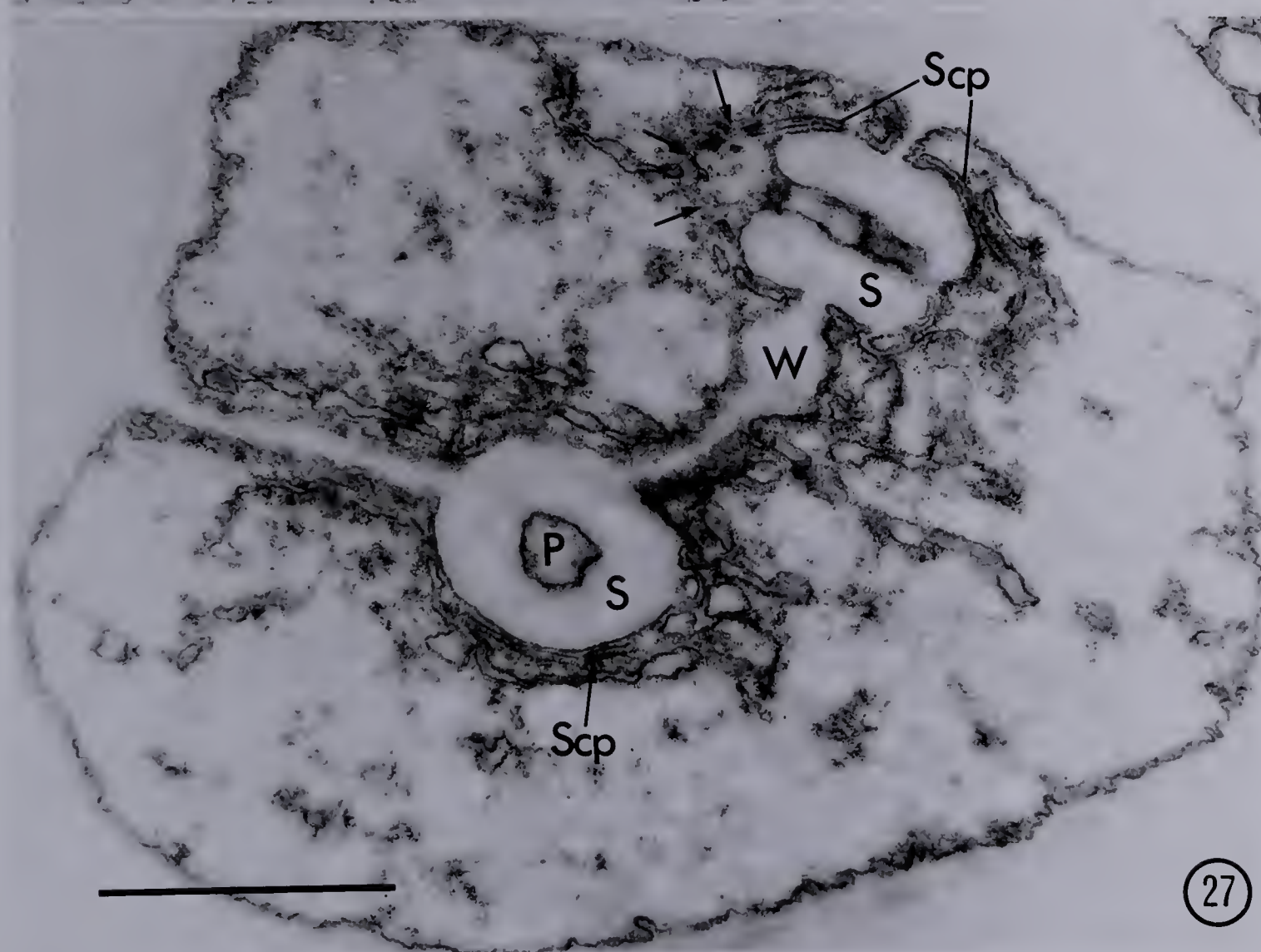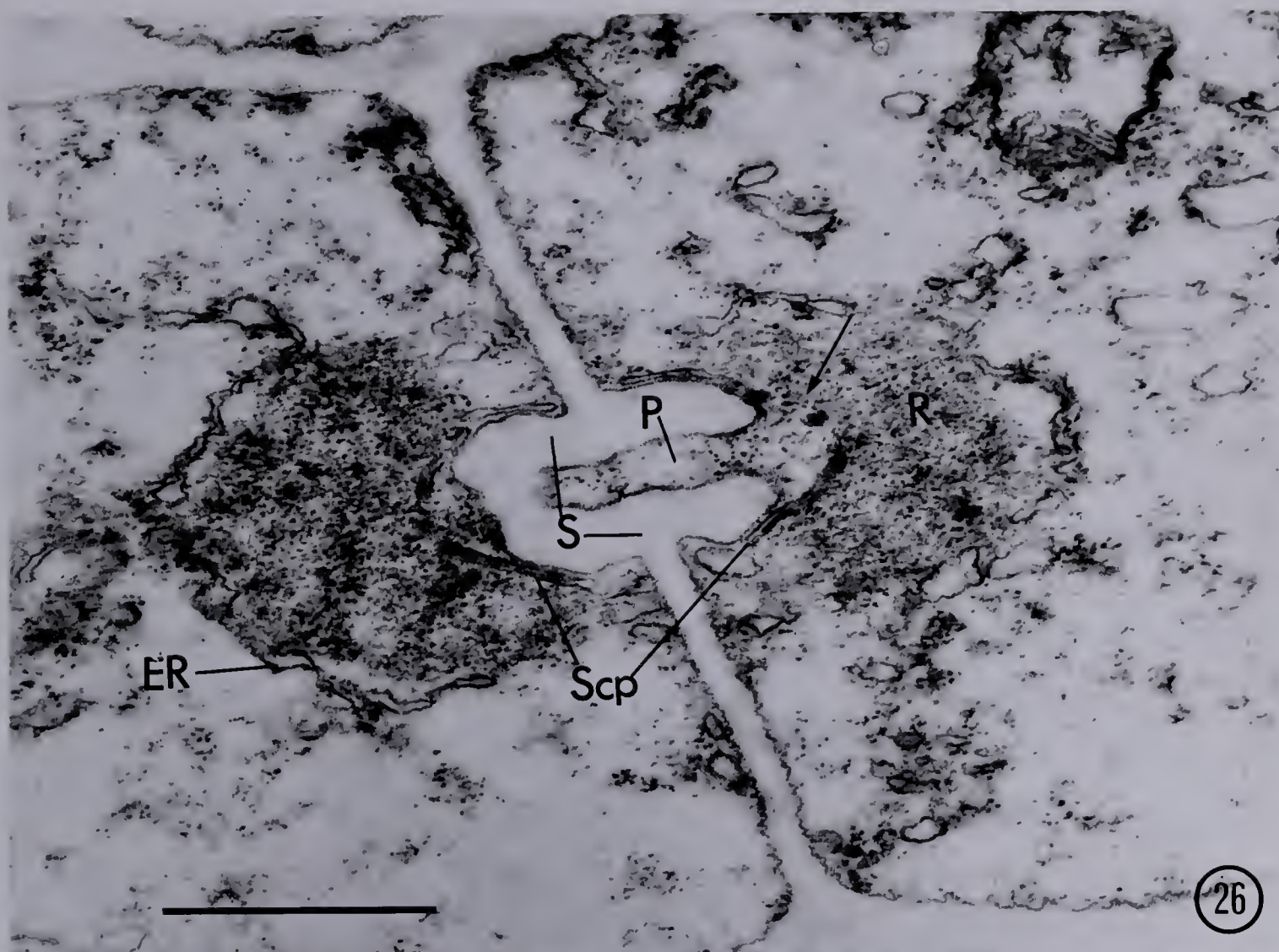
cap is perforated (like a sieve plate). Through the perforations,
cytoplasm may stream to the adjacent cell (Fig. 27, arrows). On
each side of the septal pore, there is an aggregation of ribosomes
suggesting that ribosomes may move from cell to cell. In Fig.
26 (arrow) ribosomes appear to be passing through the septal pore
and the perforation of the septal cap. The central canal of a
septal pore is about 0.25 µ and the diameter of the perforation
of the septal cap is about 0.1 - 0.2 µ.

## The Development of the Fruiting Body of Coprinus

### 1. Mycelium of Pseudoparenchymatous Tissue

Two types of mycelium have been observed in a develop-
ing fruit-body. These are termed by the author the active type
and the inactive type (Figs. 28, 29). The active type mycelium
contains an abundance of ribosomes, endoplasmic reticula, as well
as mitochondria, and nuclei rich in nucleoplasm (Fig. 28). In
contrast, the inactive type of mycelium has few ribosomes, and
has nuclei poor in nucleoplasm (Fig. 29). The sparse appearance
of the latter nuclei may result from protein starvation, as sug-
gested by Giese (1962). In this figure the chromatin network
of the nucleus is easily discerned. Similar nuclei have been
observed in cystidia, another type of inactive cell found in
Coprinus fruiting bodies.

In nuclei of inactive cells, individual chromatin
cores may be clearly identified. An individual core measures

Figs. 28 and 29. Mycelium from pseudoparenchymatous
tissue: Fig. 28, active-type cell, showing
rich content of subcellular particles;
Fig. 29, inactive-type cell, showing poor
content of subcellular particles; N, nucleus;
Nu, nucleolus; cf, chromatin fibrils; ER,
endoplasmic reticulum; R, ribosomes; Lo,
lomasome; M, mitochondria; Vc, vesicles;
W, cell wall. Magnification approximately
19,400 X.

28



29
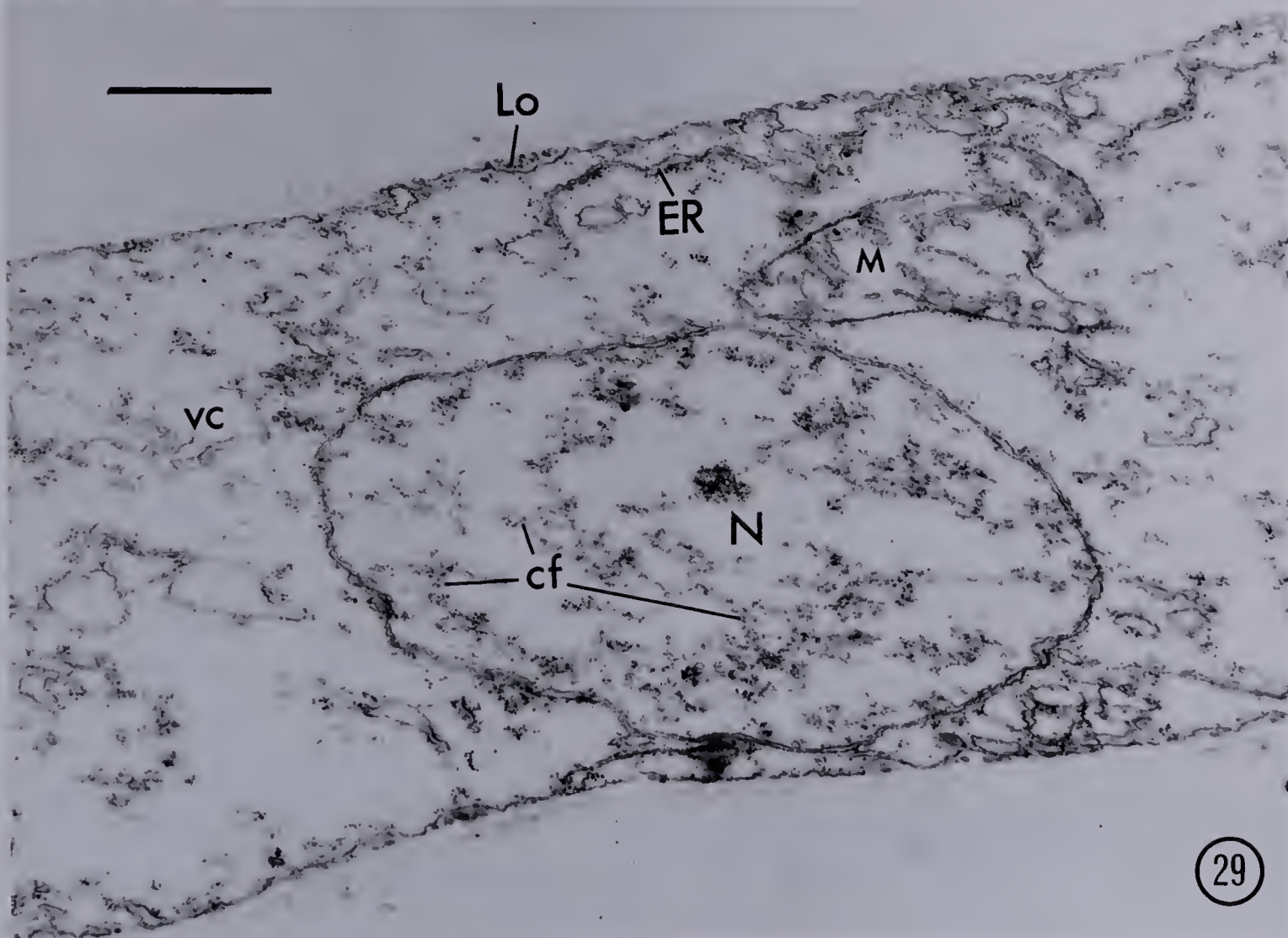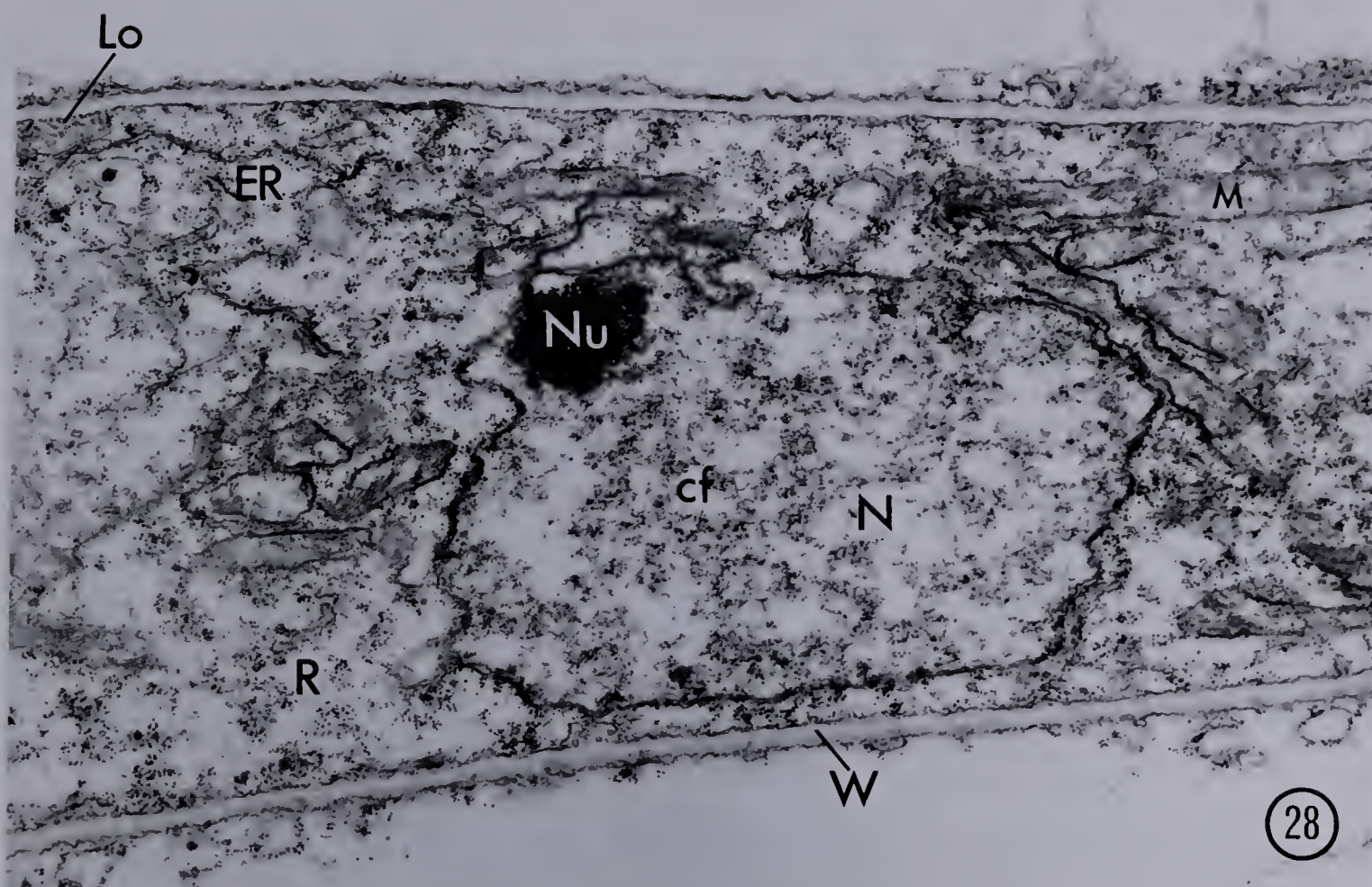
about 0.1 μ in thickness. The chromatin cores may represent
the chromosomes of interphase nuclei.

The active hyphae in the hymenium are directly con-
nected with basidia. Such hyphae are rich in stored food materials
(Fig. 32). Although such food particles are found in a young
basidium, they are rarely found in a basidium later in its develop-
ment (compare Figs. 49 and 50). This suggests that as a basidium
develops, food is supplied by the gradual mobilization of stored
food from adjacent cells.

## 2. Cystidia and Sterile Cells

The cystidia of Coprinus are giant cells which serve
as pillars to keep adjacent gills from coming into close contact.
At meiotic prophase I, cystidia resemble the inactive mycelium
in having few ribosomes. However, mitochondria are rather numerous
in them (Fig. 30). Such micromorphology suggests that cystidia
are not active in protein synthesis, but are nevertheless active
in respiration and oxidative metabolism. By the time meiosis is
complete, the cystidia have become vacuolate, and mitochondria
appear to have decreased in number (Fig. 31).

At the spore producing stage, there is an abrupt
morphogenetic change in the cytoplasm of the sterile cells sur-
rounding the basidia, presumably as the result of the change of
cellular metabolism. This is marked by the presence of numerous
osmiophilic granules (Fig. 63). What these granules are and

Figs. 30 and 31. Cystidium of Coprinus lagopus. Fig.

30, cystidium from a fruit-body, the diploid

nucleus of the basidium of which is at meiotic

prophase. Fig. 31, cystidium from a fruit-

body, in which meiosis is complete. Note

the large vacuole (V). Bas, basidium; M,

mitochondria; ER, endoplasmic reticulum;

V, vacuole; W, cell wall; N, nucleus.

Magnification approximately 4,000 X.

Bas

M

V

ER

30

W

N

V

31
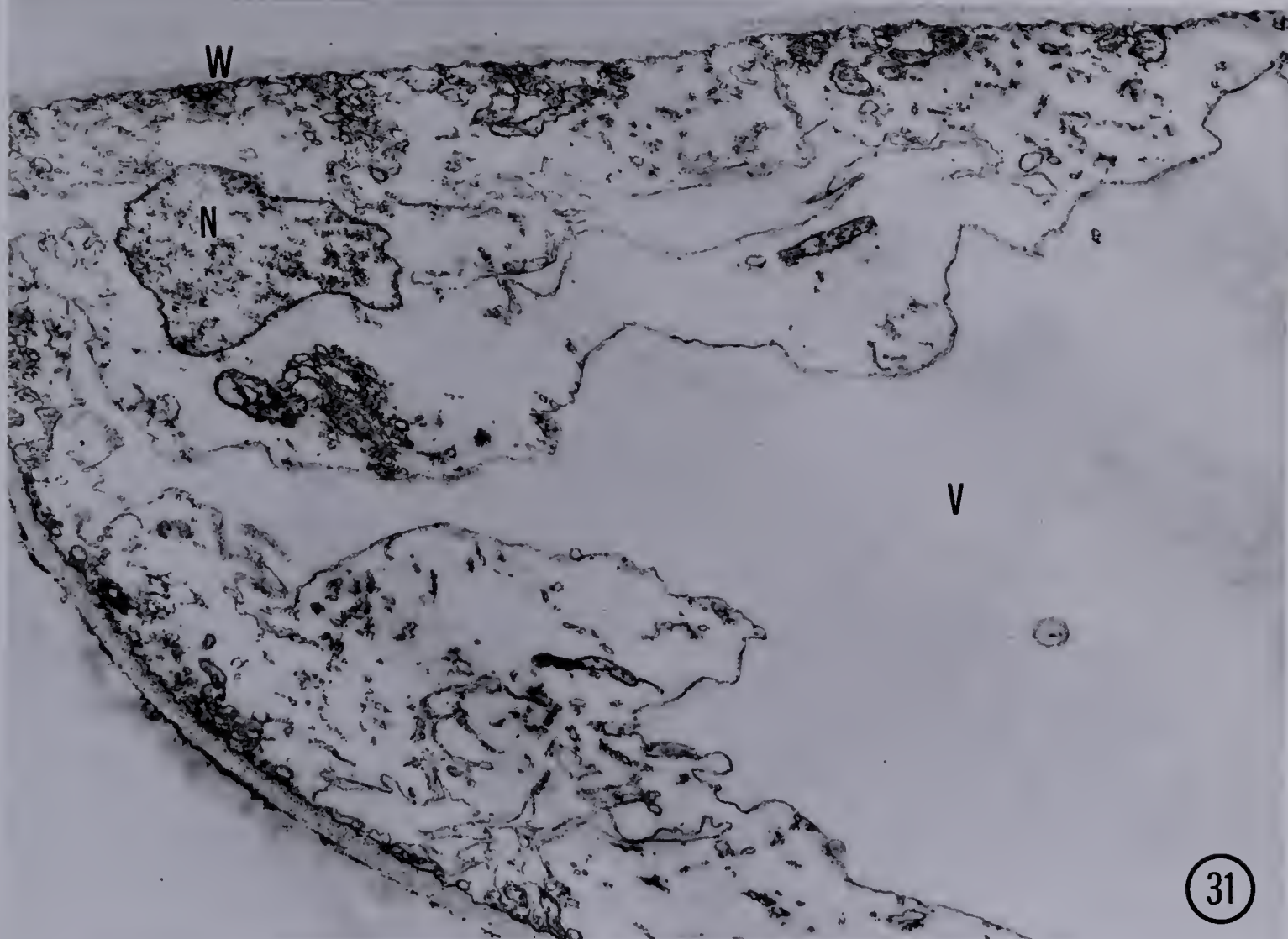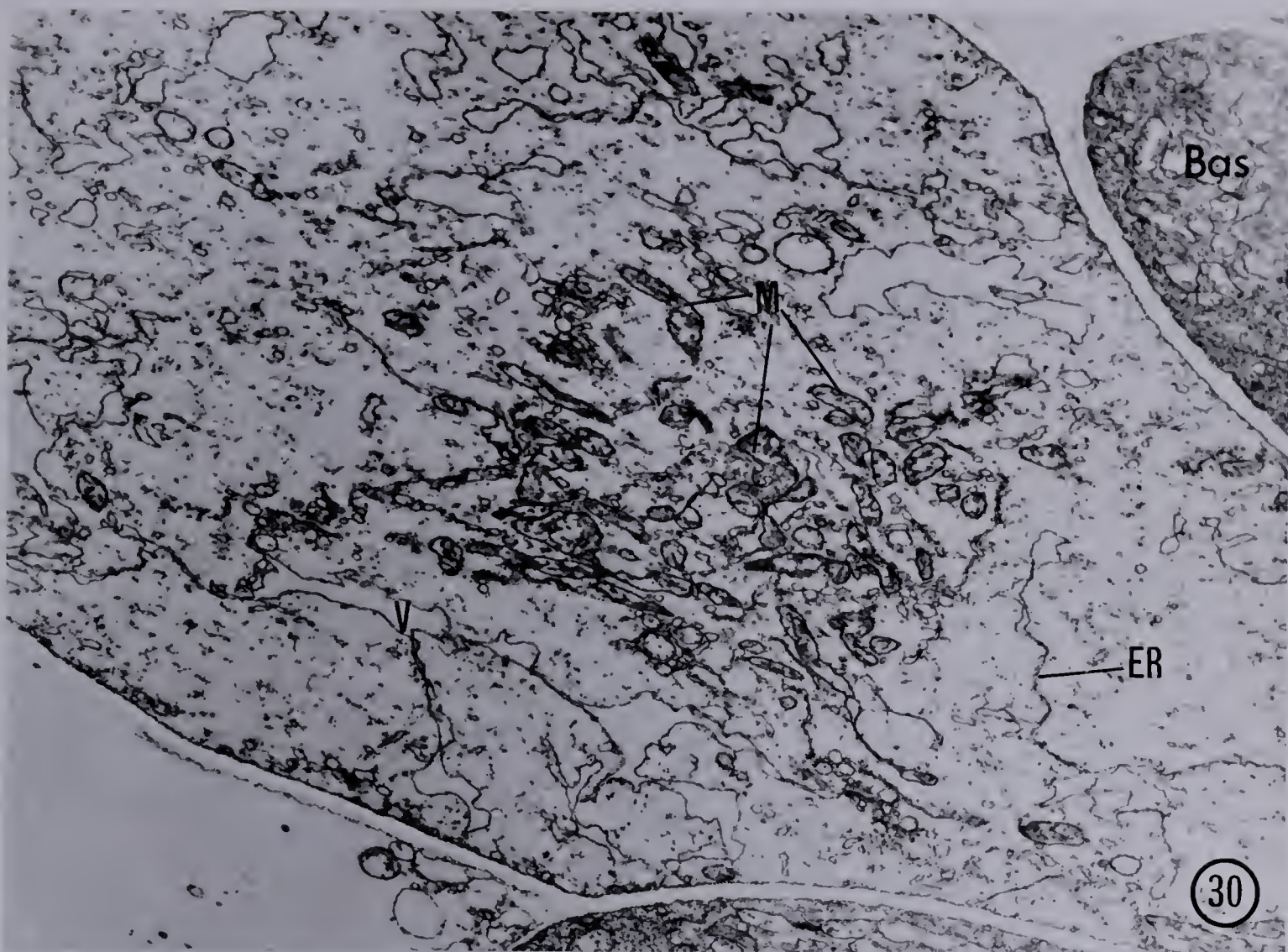
what might be their relation to development are problems that remain to be investigated.

### 3. The Basidium

In a developing fruit body, all cellular activities are channelled to, and embodied in, the basidium, in which meiosis takes place, and upon which basidiospores are produced. From the presynaptic to the pachytene stage, and thence through the second meiotic division, the basidia are abundantly provided with cellular organelles, much more so than other cell types in a fruit body.

The basidium of Coprinus lagopus arises either directly from a particular cell (hereafter it will be called the generative cell) of a hymenium, or from a stalk cell, which branches off from the generative cell (Fig. 32). Although the basidia of C. lagopus are biseriate (Buller, 1931), whether the basidium is formed from the generative cell or from the stalk cell bears no direct relationship to the biseriation. As shown in Fig. 32, both the generative and the stalk cells have abundant food particles while the basidia have none, except in the early stage of basidial development (Fig. 49).

Apart from the nucleus, the fine structure of the basidium, insofar as the cellular organelles are concerned, remains consistently dense throughout the process of meiosis. An exception is noted concerning the Golgi apparatus, the vacuoles,

Fig. 32.　A portion of the hymenium of <u>Coprinus</u> <u>lagopus</u>,

showing a generative cell (Gen) from which

a basidium (Bas) and a stalk cell (St) have

been formed; S, septum; Fd, food particles.

The arrow points to a chain of ribosomes

(polyribosome) transferring through the

septal pore.　Magnification approximately

24,000 X.
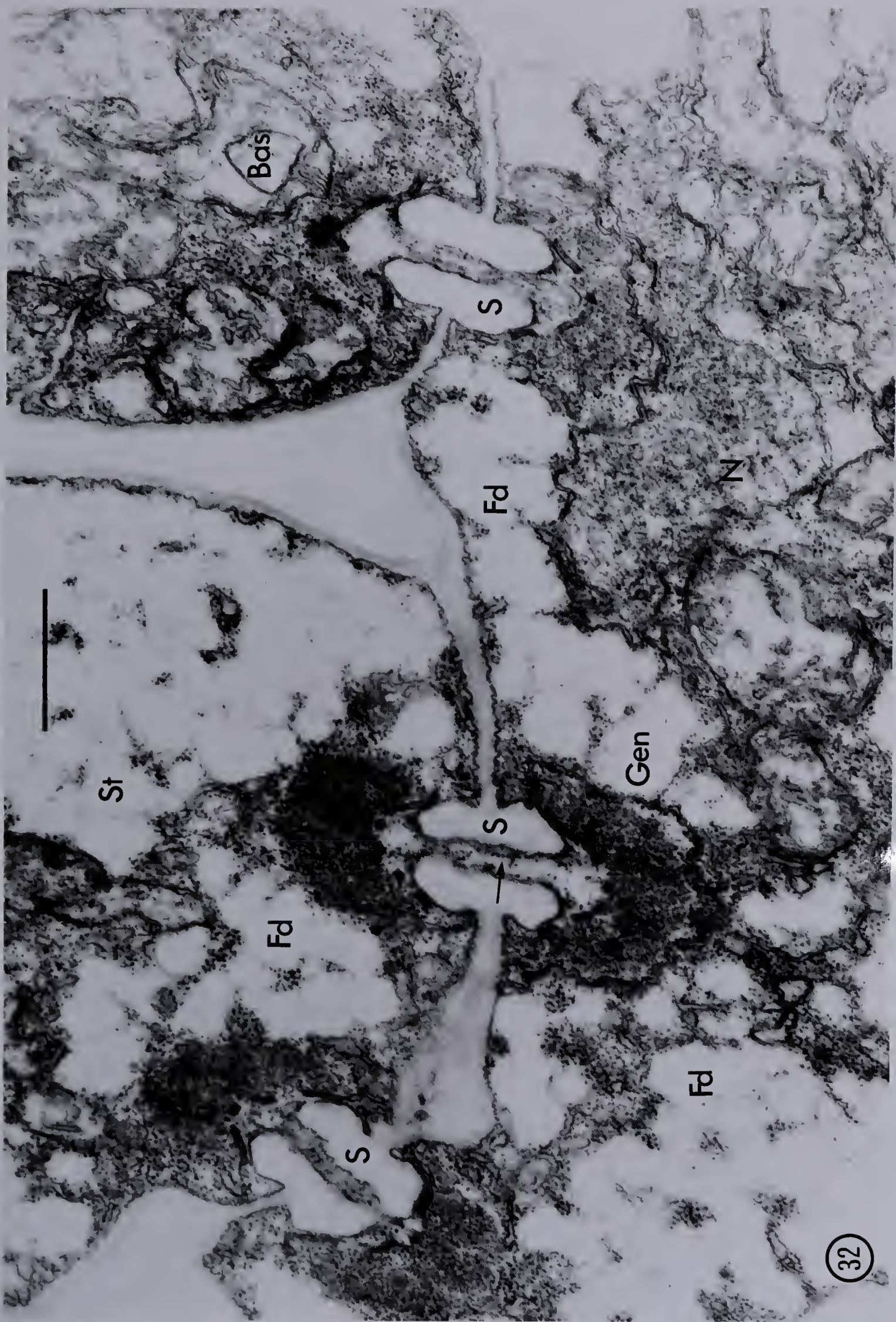
and the lomasomes. The changes undergone by these organelles have been described earlier.

## Meiosis

The following is a comparative description of nuclear fusion and meiosis as revealed jointly by light microscopy and electron microscopy.

Before nuclear fusion, a basidium contains two sexually compatible nuclei. As can be seen in Fig. 33, these have fine-stranded chromosomes typical of late telophase-interphase nuclei. In the electron micrograph shown in Fig. 49, it appears that each of these nuclei is enveloped by a double nuclear membrane. Inside each nucleus, there are a prominent nucleolus and electron-dense, evenly-distributed chromatin fibrils. The latter appear to have the same width as those to be described for later division stages.

The fusion nucleus was studied both with the light microscope (Fig. 34) and with the electron microscope (Fig. 50). In Fig. 50, the nuclear membrane is shown to be incompletely closed (large arrow), and the nucleoli to have not yet fused. There is a tripartite structure (small arrow), the details of which will be described later. The presence of this, according to the view currently held dealing with higher plant and animal cells (Ris, 1961; Moses and Coleman, 1964), indicates that synapsis has probably begun. The details of the synaptic process

Figs. 33 - 38. Synaptic stages of Coprinus comatus:

Fig. 33, two nuclei before fusion; Fig. 34,

fusion nucleus where two nucleoli have not

yet fused; Fig. 35, synaptic stage; Fig. 36,

synaptic stage, note the chromosome threads

as thick as those of Fig. 33; Fig. 37,

pachytene (synapsis complete); Fig. 38,

diplotene, note that the two homologous

chromosomes are wide apart.  Magnification

approximately 3,000 X.


Figs. 39 and 40.  Meiotic chromosomes of Coprinus

lagopus:  Fig. 39, pachytene, two homologous

chromosomes are closely appressed; Fig. 40,

diplotene, the two largest chromosomes

exhibit coiling (or folding) of chromone-

mata.  Arrows mark the acrocentric constric-

tion.  Magnification approximately 3,500 X.

10 JJ
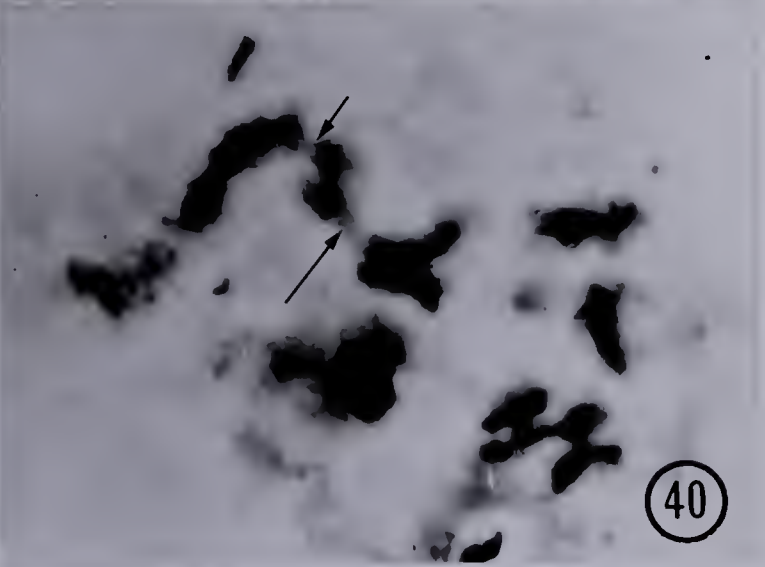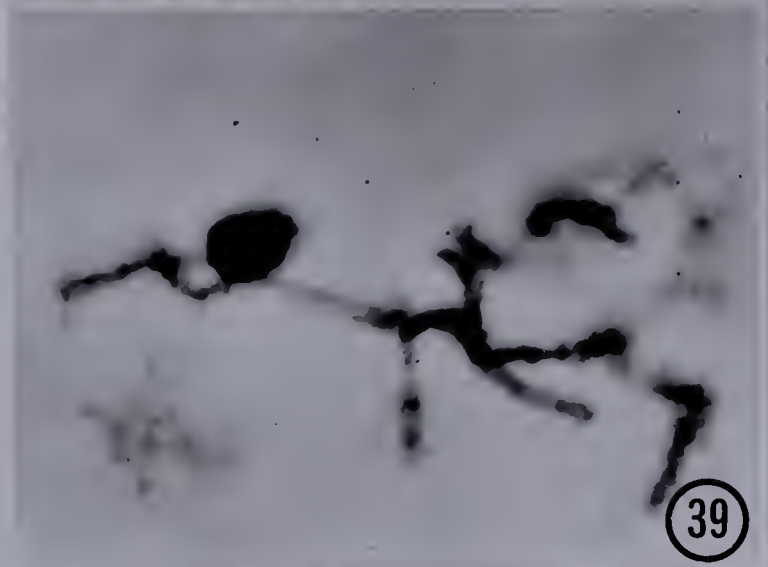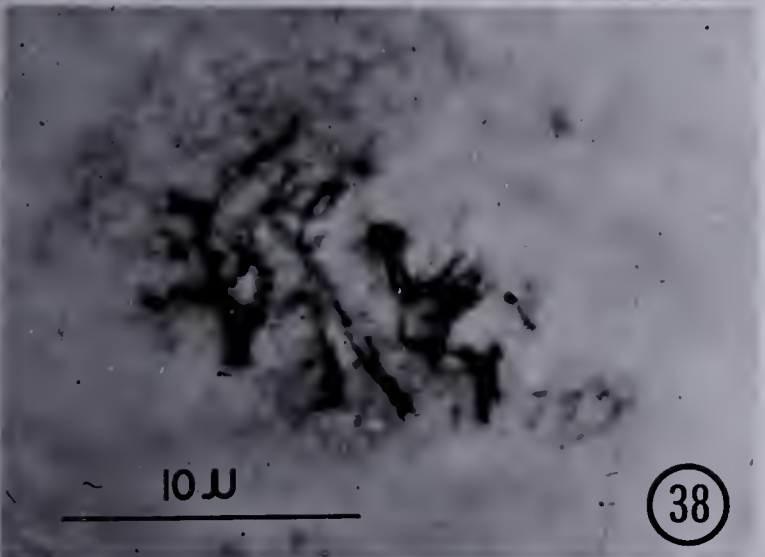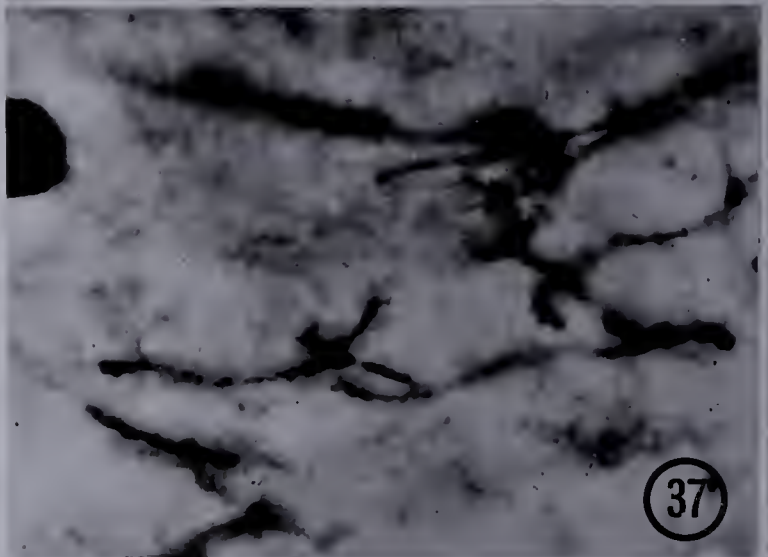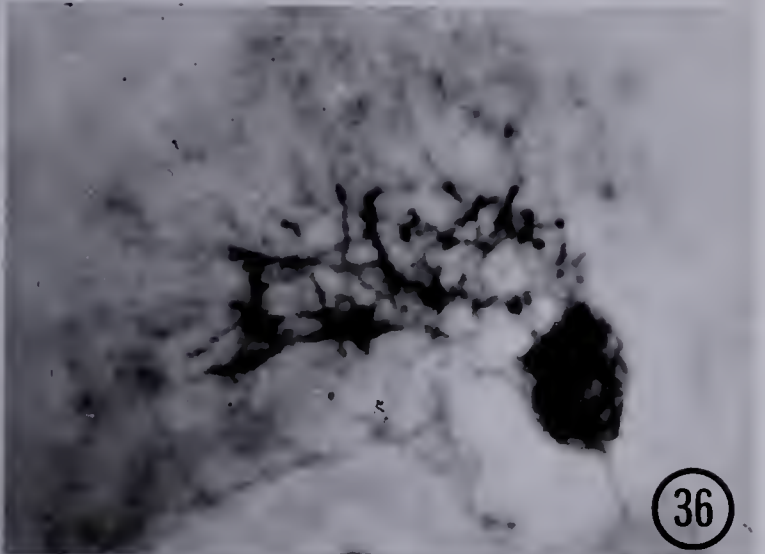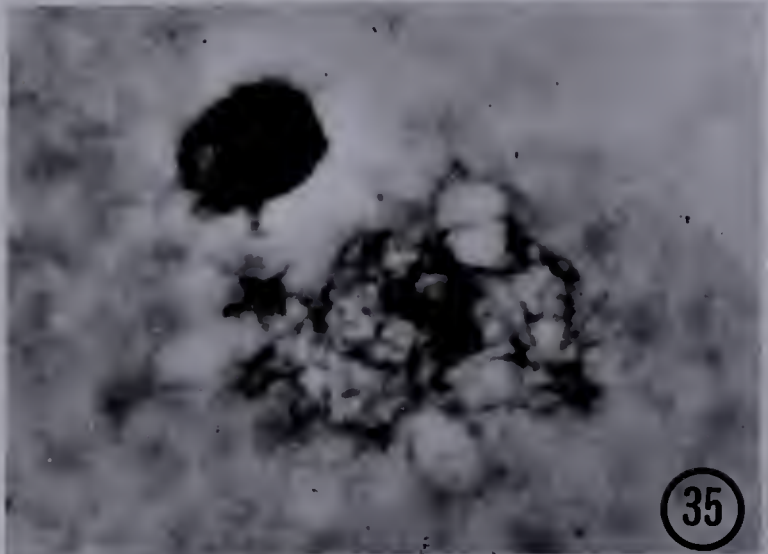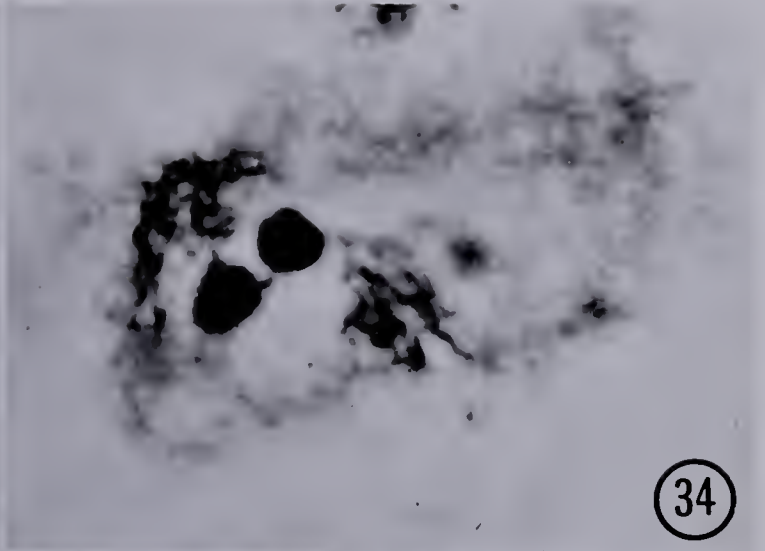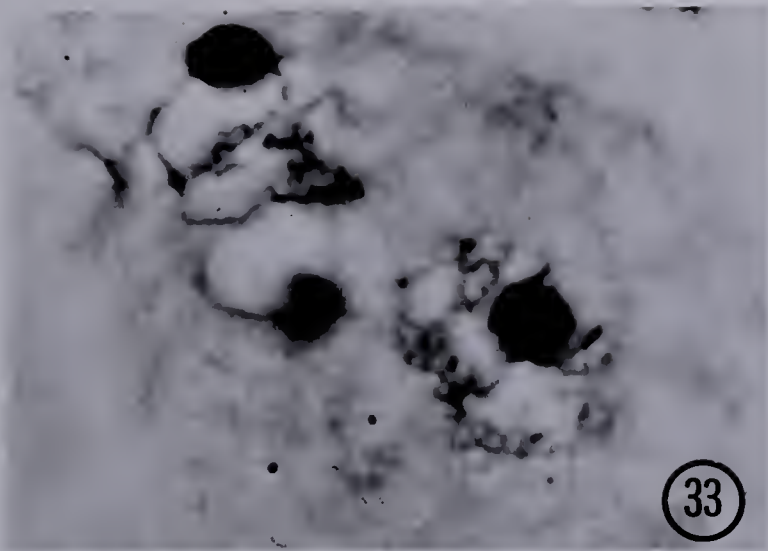
Figs. 41 - 48.  Meiotic stages of Coprinus lagopus:

Fig. 41, diplotene showing ten chromosomes.

The two largest ones show end-to-end assoc-

iation.  Note chromosome knobs in which

lateral loops (or bristles) radiate from

parallel homologues (arrows).  Fig. 42,

diakinesis; Fig. 43, metaphase; Fig. 44,

metaphase-anaphase, showing centrioles and

spindle fibers; Fig. 45, late anaphase,

chromosomes at the poles, the structural

spindle is seen as a bridge between the

daughter-chromosome groups; Fig. 46, meta-

phase II, showing two pairs of centrioles

(large arrow indicates a centriole not in

focus); the pair at the left is associated

with the chromosome mass, the pair at the

right is not associated with the chromosome

mass; small arrows indicate structural spindles;

astral rays are evident; Fig. 47, metaphase

II, Fig. 48, tetrad (four nucleus stage).

Magnification approximately 3,500 X.

41
42

10 JU

43
44

45
46

47
48
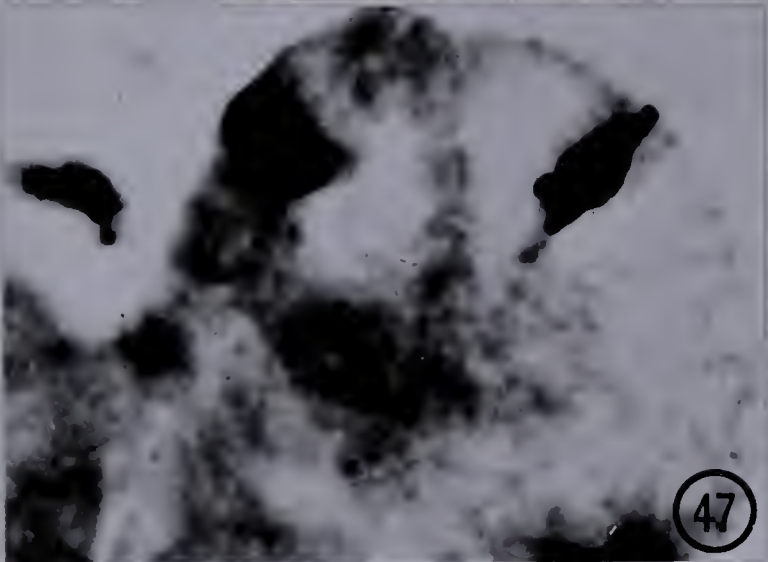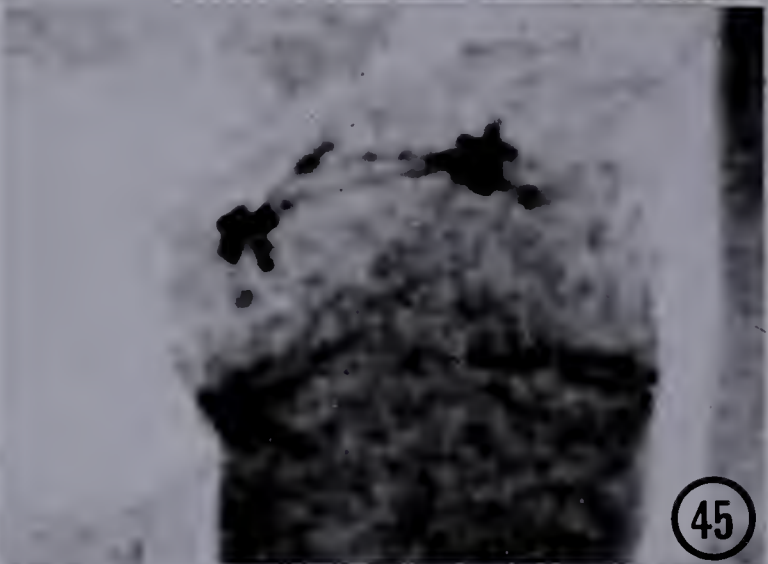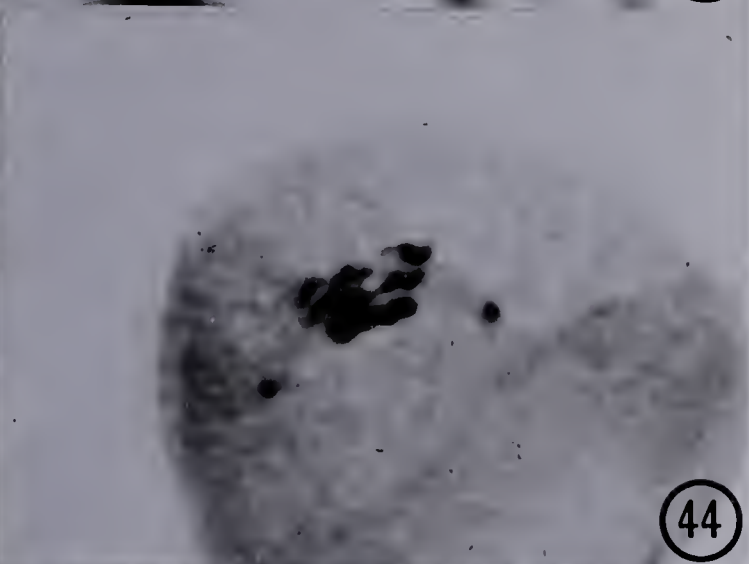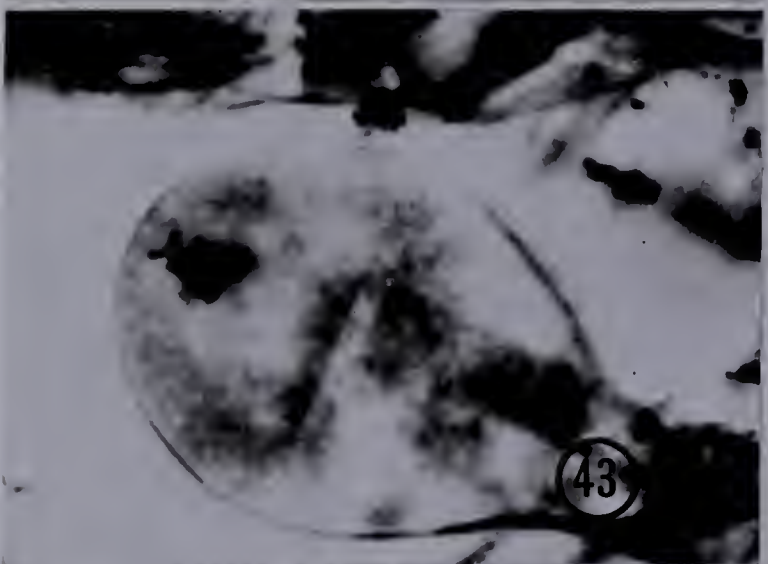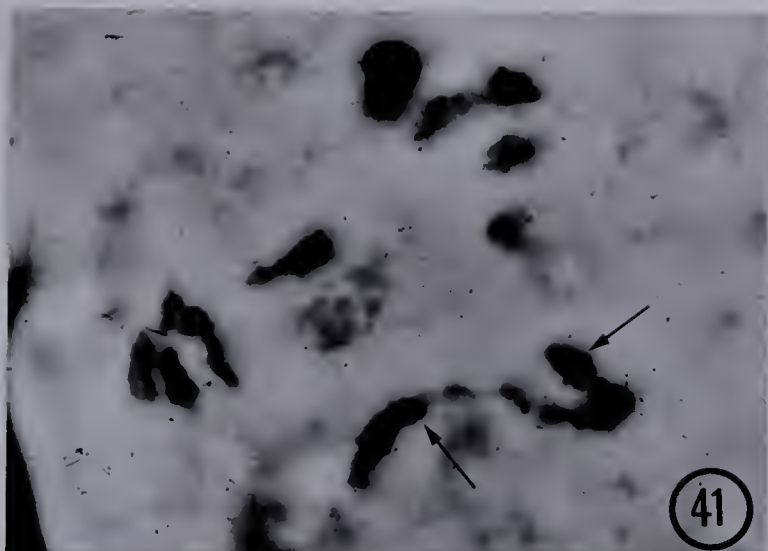
cannot be followed by the techniques used; the difficulty is probably due to the entangling of extended chromatin fibrils which, when sectioned, hamper the indentification of particular entities.

Later the nucleoli fuse as may be inferred from Figs. 35 and 36. Chromosomes appear long and thin, and consist of strands of about the same thickness as those shown in Fig. 33. It is therefore probable that synapsis is not yet completed. After synapsis, chromosomes go through pachytene (Figs. 37, 39), diplotene (Figs. 38, 40, 41), diakinesis (Fig. 42), metaphase I (Figs. 43, 44), anaphase I (Fig. 45), telophase I, and thence through the second division of meiosis (Fig. 47) to form the four nuclei of the tetrad stage (Fig. 48).

At pachytene, the two homologous chromosomes of a bivalent are closely paired, each homologue remaining discernible (Fig. 39). A longitudinal section through a bivalent at pachytene, as revealed by the electron microscope, exhibits a tripartite structure (Figs. 52, 53, 54) with all the characteristics of the synaptinemal complex of higher organisms (See Moses and Coleman, 1964 for a review of data concerning this complex). This consists of two homologous-chromosome axes about 50 mμ thick equidistant from a central linear element about 15 mμ thick.

The following terminology is defined and adopted in the present thesis and is used to describe structures such as those above. (1) <u>Synaptinemal</u> <u>complex</u> (a bivalent chromosome

at the synaptic stage): In the electron micrographs, it appears
as a tripartite structure consisting of three elements, namely,
two homologous-chromosome axes, and one synaptic center.  The
term was first suggested by Moses (1958) and has since been
adopted by most authors.   (2) Homologous-chromosome axis (the
axis of a homologous chromosome):  In the electron micrographs,
it appears as a cylinder of high electron density from which
chromatin fibrils radiate laterally.  In the literature, it has
been known as "lateral element", "lateral component", axial com-
ponent", or "chromosome core".  (3) Synaptic center: It is the
pairing surface where two homologous chromosomes meet.  It may
be the area of effective pairing where crossing-over may take
place.  In the literature, it has been known as "central element",
central component", central filament", or "mid-axial element".

At diplotene, when bivalents are condensed (Fig. 38);
at this stage, the nucleolus of C. comatus appears to decrease in
size.  A longitudinal section of these examined by means of the
electron microscope (Fig. 57) reveals a chromosome morphology
comparable to that seen under the light microscope (Fig. 38).
Fig. 57 depicts a section of the nucleolus chromosome.  The
nucleolus-organizer parts of the two homologues appear to be
embedded in the nucleolus.  Also the two homologues are far
apart.  This is in agreement with what has been observed with
the light microscope (Fig. 38).

As shown in Fig. 40 (arrow), coiling of the chrom-
onemata may be inferred from the shape of the two largest

Fig. 49.  Basidium of <u>Coprinus</u> <u>lagopus</u> showing two

compatible nuclei before fusion.  M, mito-

chondria; N, nucleus; Nu, nucleolus; R,

ribosomes; Fd, (presumably) food particles;

S, septum; W, cell wall; St, stalk cell.

Magnification 26,000 X.
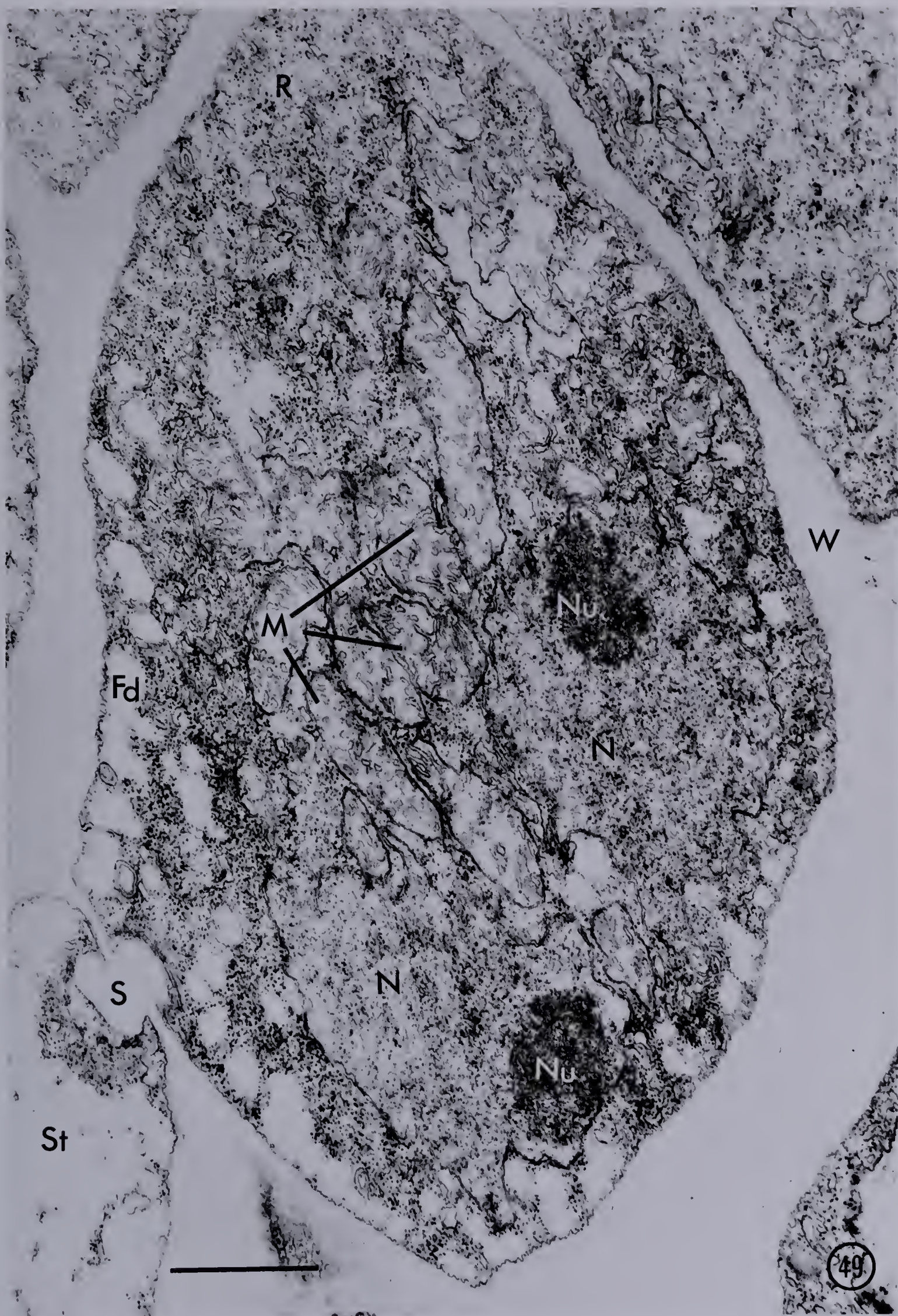
Fig. 50.  Basidium of _Coprinus lagopus_, showing fusion
          nucleus at synaptic stage; the nuclear mem-
          brane is not completely closed (large arrow);
          two nucleoli have not yet fused; Syn, synaptin-
          emal complex; N, nucleus; R, ribosomes; ER,
          endoplasmic reticulum; cf, chromatin fibrils.
          Magnification approximately 33,000 X.
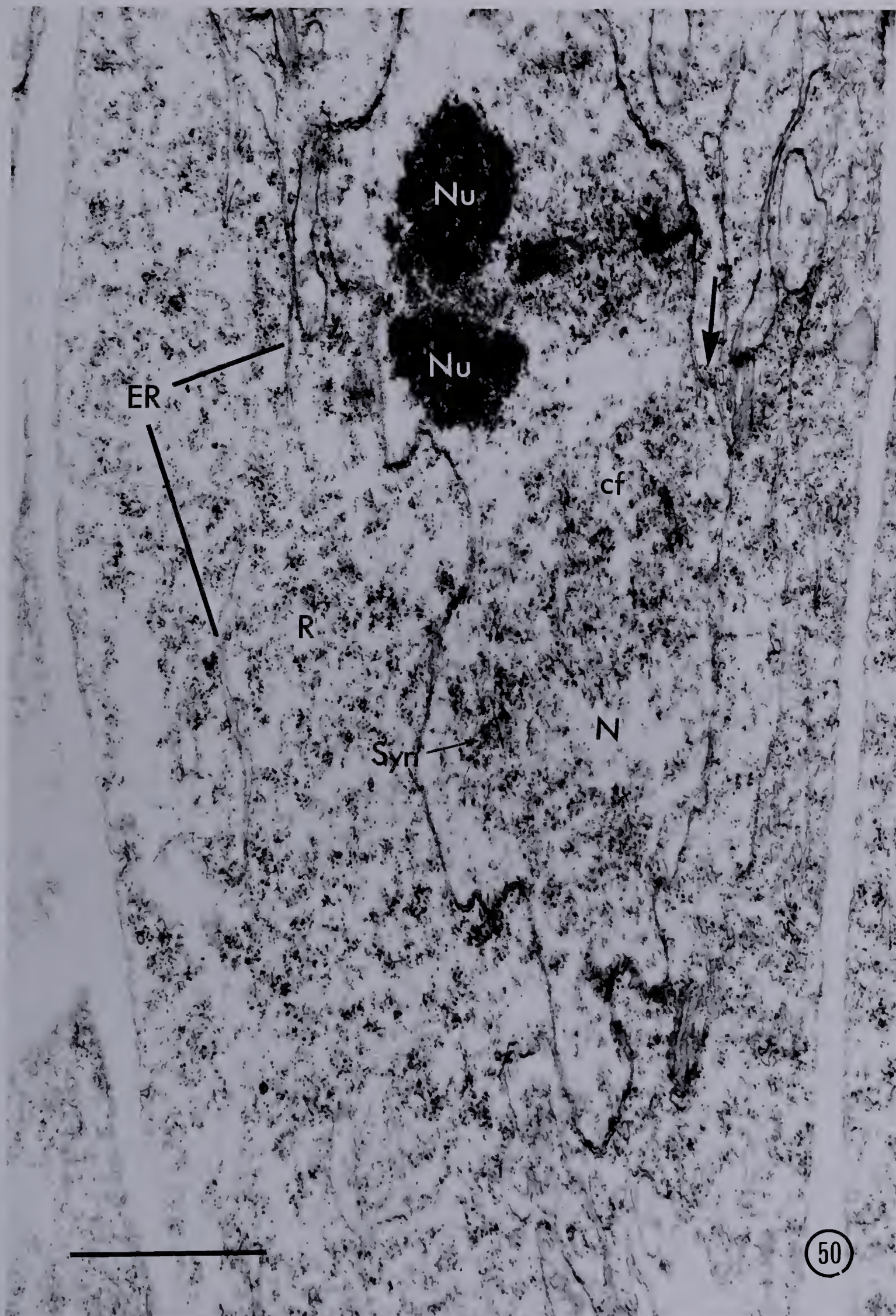
Fig. 51.  Basidium of <u>Coprinus</u> <u>lagopus</u>, showing the

diploid nucleus at meiotic prophase; note

mitochondria surrounding the nucleus.  N,

nucleus; Nu, nucleolus; cf, chromatin fibrils;

Ca, chromosome axes; M, mitochondria; W,

cell wall.  Magnification approximately

15,000 X.


Fig. 52.  Basidium of <u>Coprinus</u> <u>lagopus</u>, showing the

diploid nucleus at meiotic prophase; note

continuity of chromosome (Ch); N, nucleus;

V, vacuole; M, mitochondria; W, cell wall.

Magnification approximately 17,000 X.

Figures 51 and 52. Labels: Nu, cf, N, Ca, M, W, Ch, V.
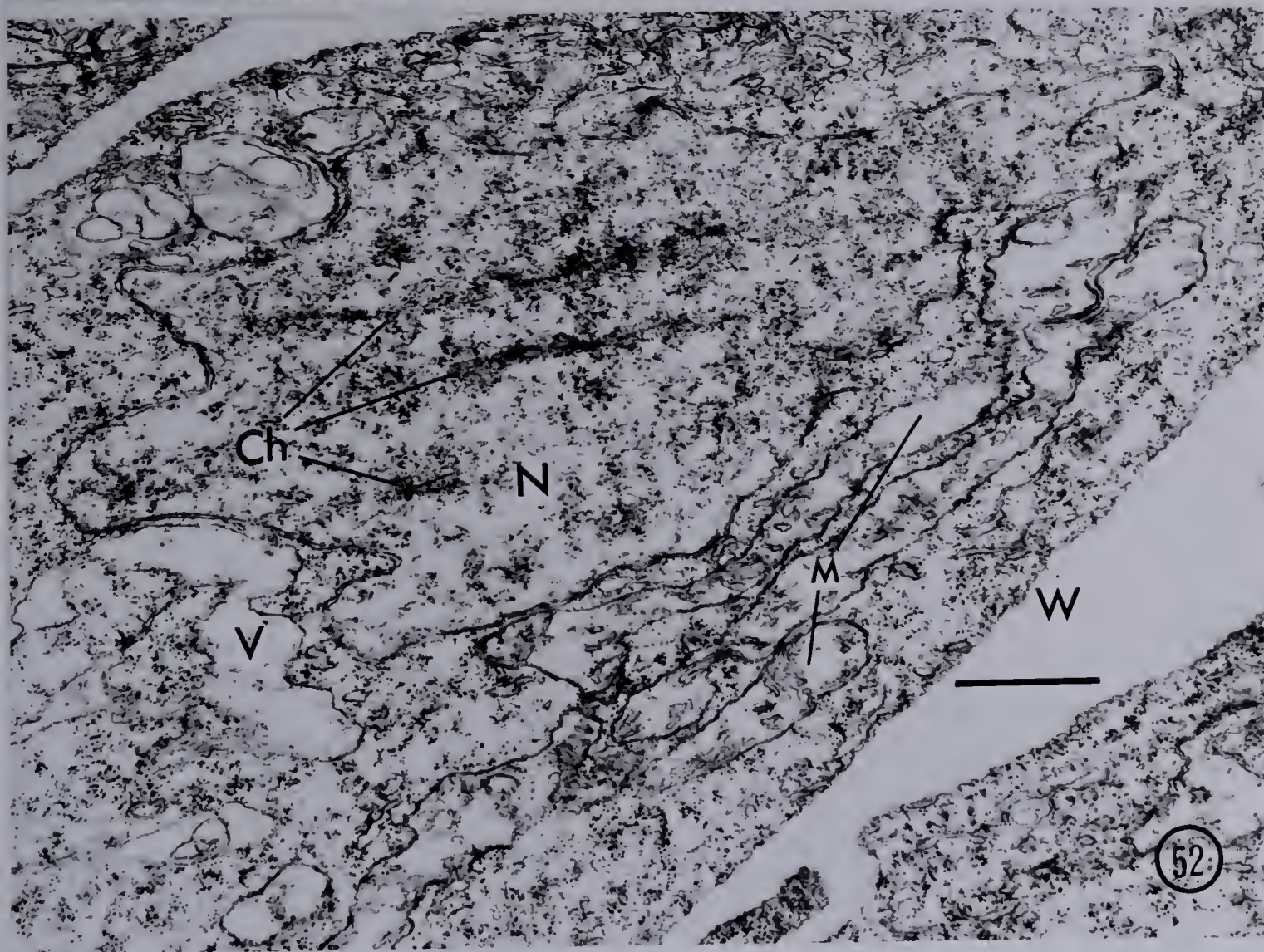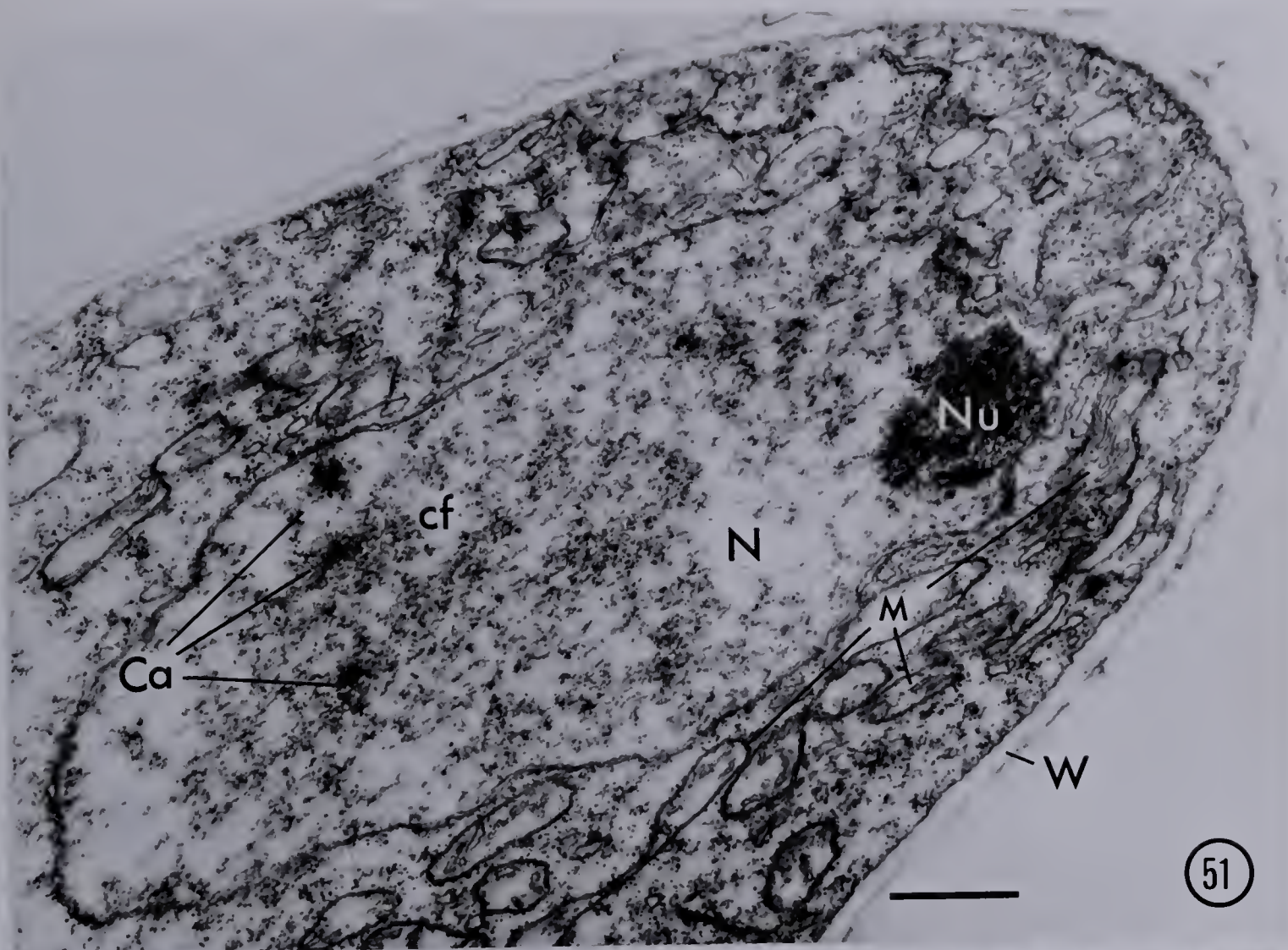
Fig. 53.  Diploid nucleus of _Coprinus_ _lagopus_ showing;
         Syn, synaptinemal complex; N, nucleus; ba,
         the synaptic center (or bivalent axis); ca,
         the homologous-chromosome axis; R, ribosomes;
         M, mitochondria.  Magnification approximately
         68,000 X.


Fig. 54.  A synaptinemal complex (Syn):  The synaptic
         center (ba) is prominent.  Arrows mark the
         homologous-chromosome axes from which lateral
         projections radiate.  Magnification approx-
         imately 91,600 X.
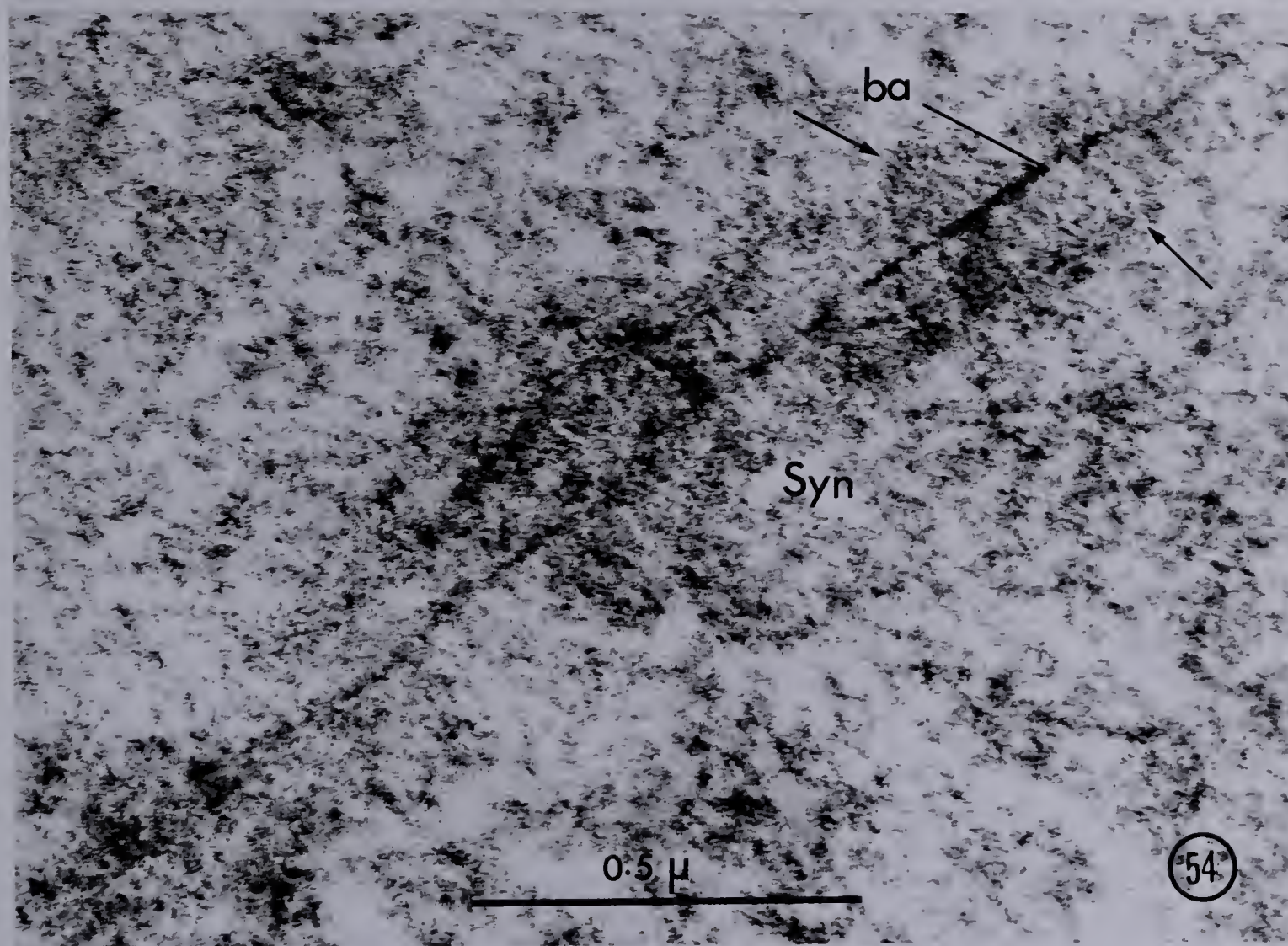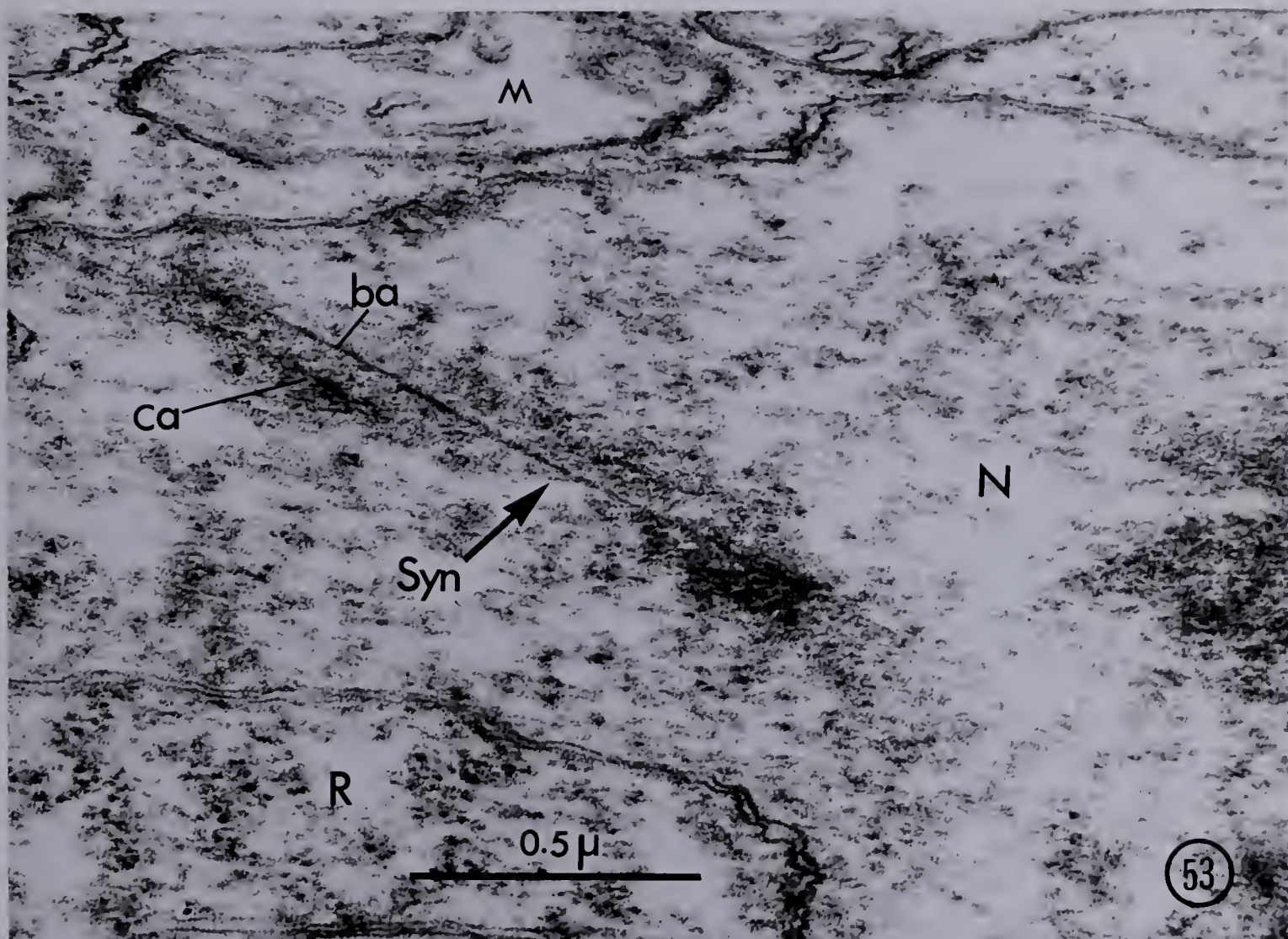
chromosomes. The chromatin materials appear to be clustered
(Fig. 41).

At metaphase, chromosomes are very condensed and are
gathered at the equatorial plate (Fig. 43). They are held by
the spindle fibers to a pair of centrioles (Fig. 44). Follow-
ing metaphase I, the basidium goes through the two-nucleus
stage (Fig. 59), and thence the four-nucleus stage (Figs. 48,
60,61). The electron microscope shows that each nucleus at the
two-nucleus stage as well as at the four-nucleus stage is en-
veloped by a double nuclear membrane. The nucleoplasm exhibits
the even texture common to all interphase nuclei. As illus-
trated in Fig. 44 and inferred from Fig. 59, the first meiotic
spindle is oriented across and is near the apex of the basidium.

## Centrioles and Spindle Formation

Centrioles and spindle fibers have been observed by
means of classical cytology (Fig. 44). This observation poses
a number of questions. How, for example, is the centriole
associated with the nucleus, especially when the latter can
migrate from cell to cell in the mycelium? When does the cen-
triole divide in relation to the chromosome cycle?

In Fig. 13, a centriole is seen at the pole just before
metaphase I. It is evident that it is made up of tubules. In
Fig. 58, a section of a centriole is seen in association with
the spindle fibers, which were cut obliquely. Some astral rays
are discernible. The membrane, partly surrounding the spindle
zone, may probably be the nuclear membrane. It is suggested

Figs. 55 and 56.  Diploid nuclei of Coprinus lagopus,

showing the association of the centriole

with the outer nuclear membrane:  In Fig.

55, the centriole is dividing.  The large

arrow marks the overlapping of the nuclear

membrane.  In Fig. 56, small arrows mark

the 100-Å-thick fibrils resulting from the

coiling of a 20-30 Å thick subunit.  N,

nucleus; cf, chromatin fibrils; R, ribo-

somes.  Magnification approximately 60,000

X.
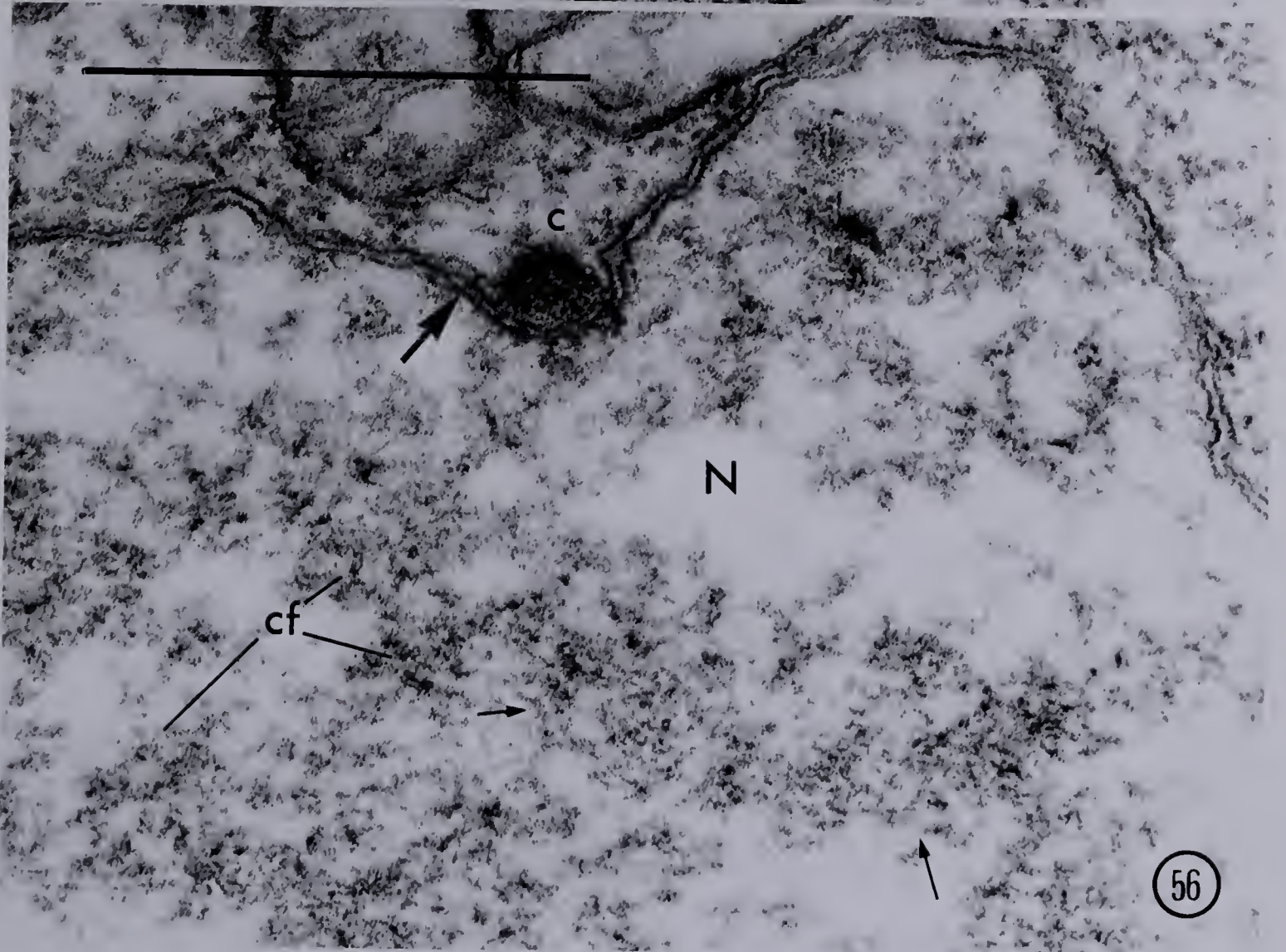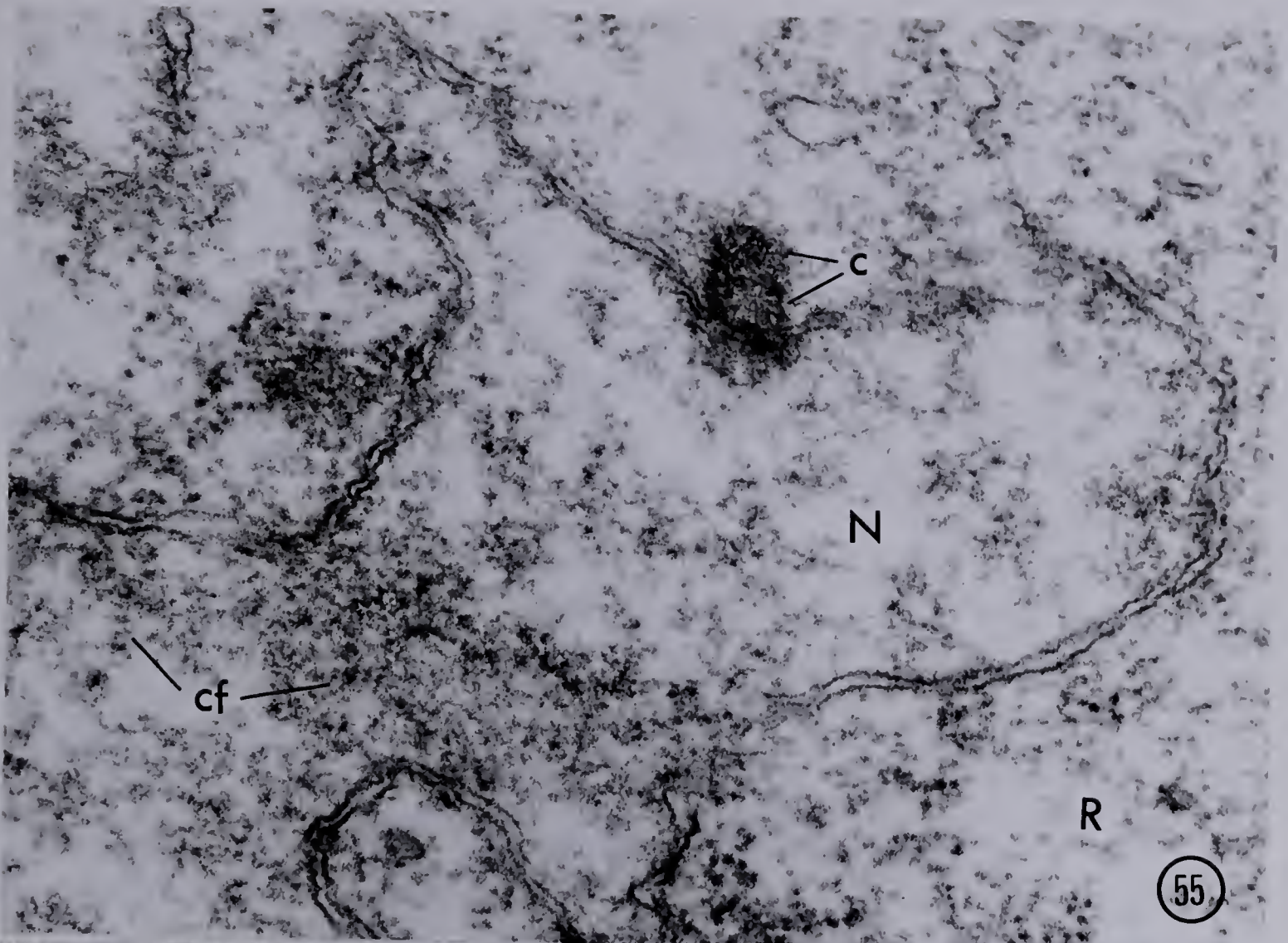
that the nuclear membrane may be partly dissolved at metaphase.

In Fig. 55, and again in Fig. 56, there is shown a very electron-dense structure in association with the outer nuclear membrane. This granule is often seen sunken in the nuclear invagination as shown in Fig. 69. This structure has been observed repeatedly in the electron micrographs. As shown in Fig. 56, the nuclear membrane near this structure appears to be triple (arrow); apparently, this is due to the overlapping of part of the nuclear membrane. What the structure really is remains to be established. It is very osmiophilic, it is ovoid, and it appears to possess structural organization (Fig. 55) which might be tubules as shown in Fig. 13. In addition, it appears to be dividing when the nucleus is in meiotic pro-phase (Fig. 55). These observations together with the asso-ciation of the ovoid body with the outer nuclear membrane strongly suggest that it may be the centriole.

If this can be established, it follows that the cen-triole of higher fungi is intimately associated with the outer nuclear membrane. Such an association has been observed under the light microscope in the mycelium of Cyathus (Lu, 1964a) and in Neurospora (Lu, 1962).

Fig. 46 represents a very interesting example of spindle formation. There are shown two pairs of centrioles with spindles between them. One pair has chromosomes attached to the spindle, the other pair has not. In the latter, the structural

Fig. 57.  Nucleus of _Coprinus_ _lagopus_ at diplotene

stage of meiosis, showing the nucleolus

chromosome; N, nucleus; Nu, nucleolus; Ch,

two homologues of the nucleolus chromosome;

cm, centromere; ER, endoplasmic reticulum

associated with the nuclear membrane.  Large

arrow marks a probable chiasma; small arrows

point to two chromatids of a homologous

chromosome.  Note, the nucleolus-organizing

parts of the chromosomes are embedded in the

nucleolus.  Magnification approximately 39,600

X.
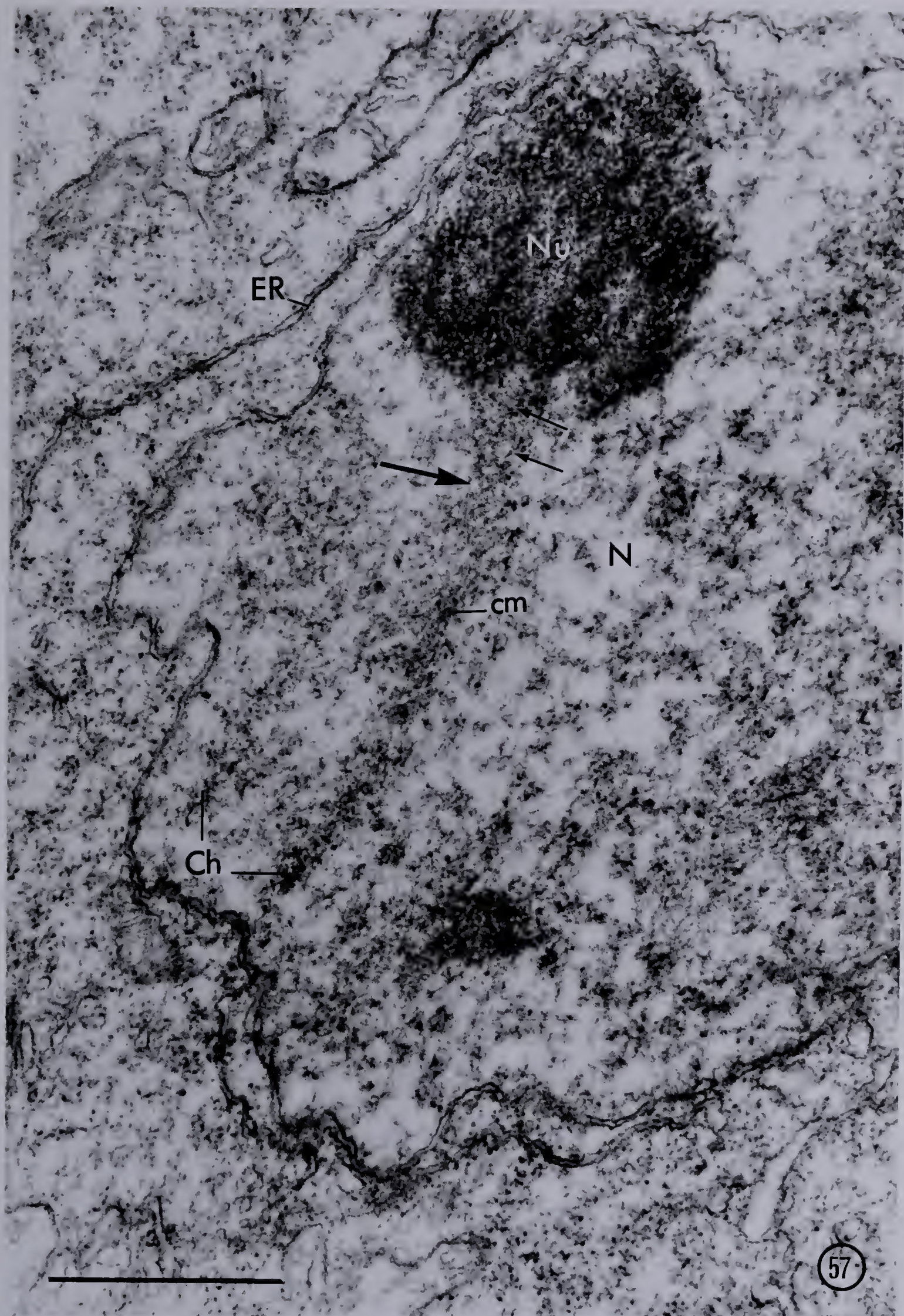
Fig. 58.   Basidium of <u>Coprinus</u> <u>lagopus</u> at metaphase I,

showing a centriole in section (c); arrow

marks the astral rays; NM, nuclear membrane;

Spz, spindle zone; M, mitochondria; GV,

golgi vesicles.   Note, Golgi vesicles in

association with the spindle zone.   Magnif-

ication approximately 23,000 X.


Fig. 59.   Basidium of <u>Coprinus</u> <u>lagopus</u> at two nucleus

stages (interphase I).   M, mitochondria;

Lo, lomasome; V, vacuole.   Magnification

approximately 23,000 X.
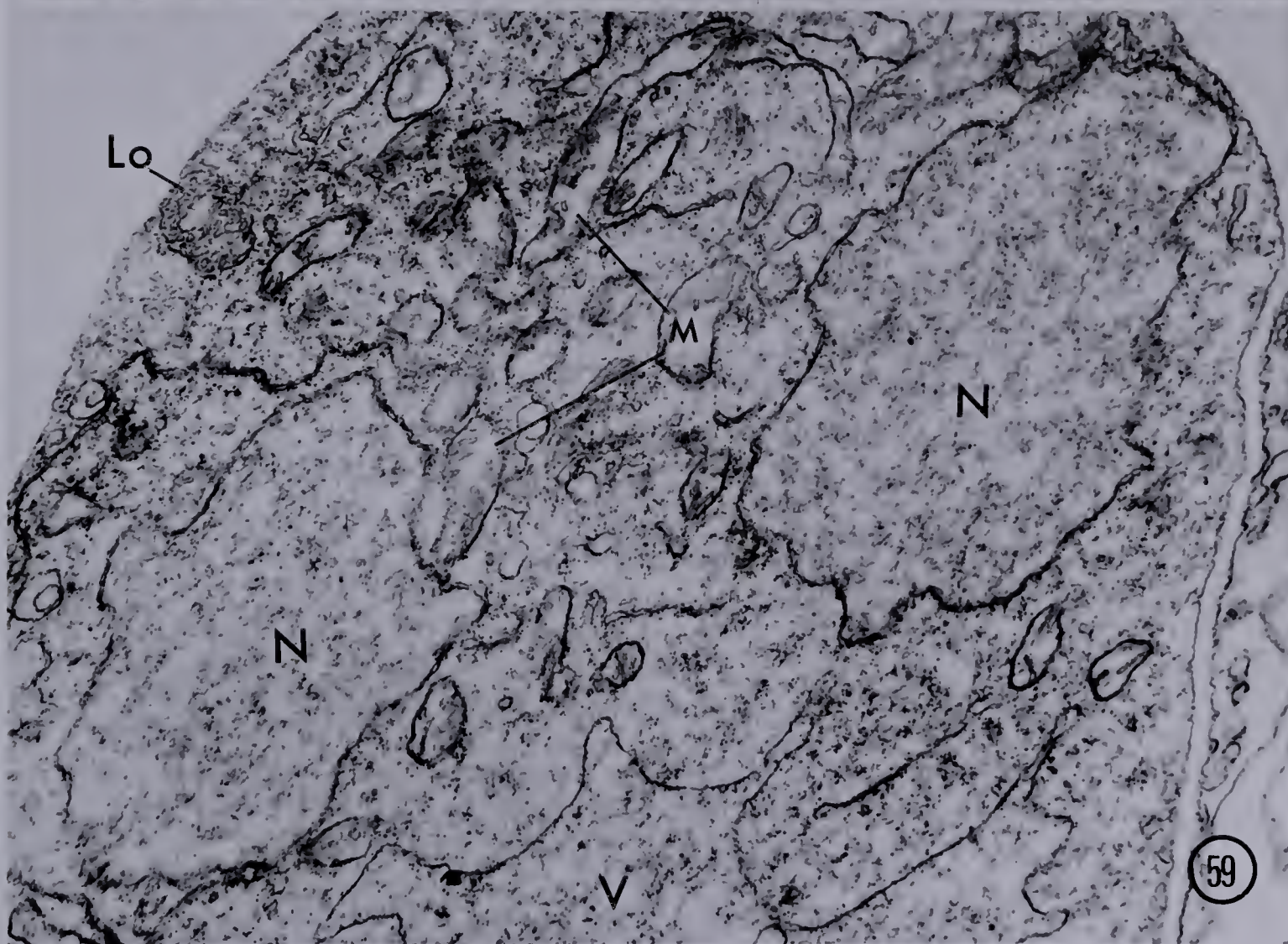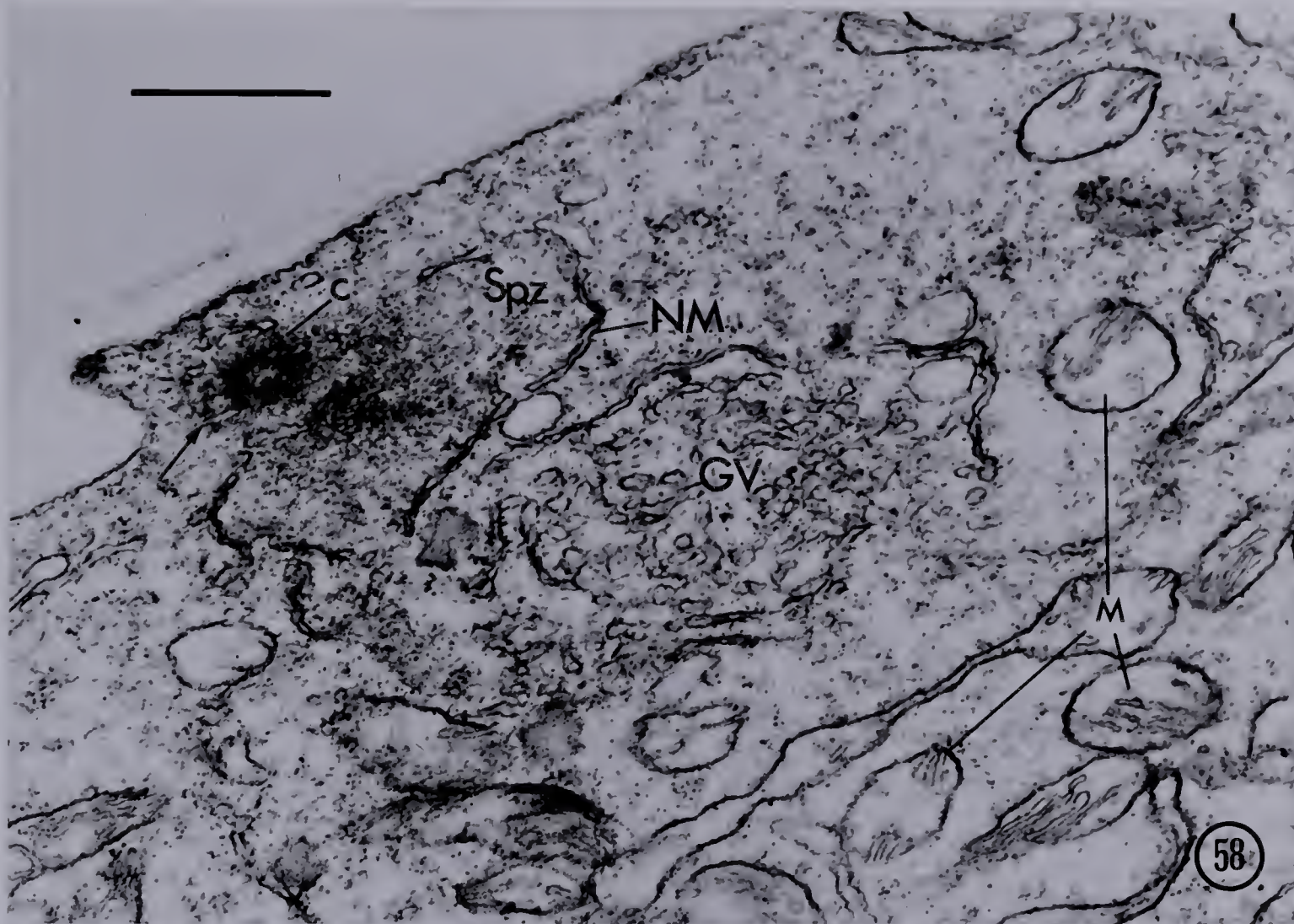
Fig. 60.   Basidium of <u>Coprinus</u> <u>lagopus</u> at tetrad stage, showing four nuclei (N); note the endoplasmic reticulum (ER) in association with the nuclear membrane.  Magnification approximately 14,700 X.

60
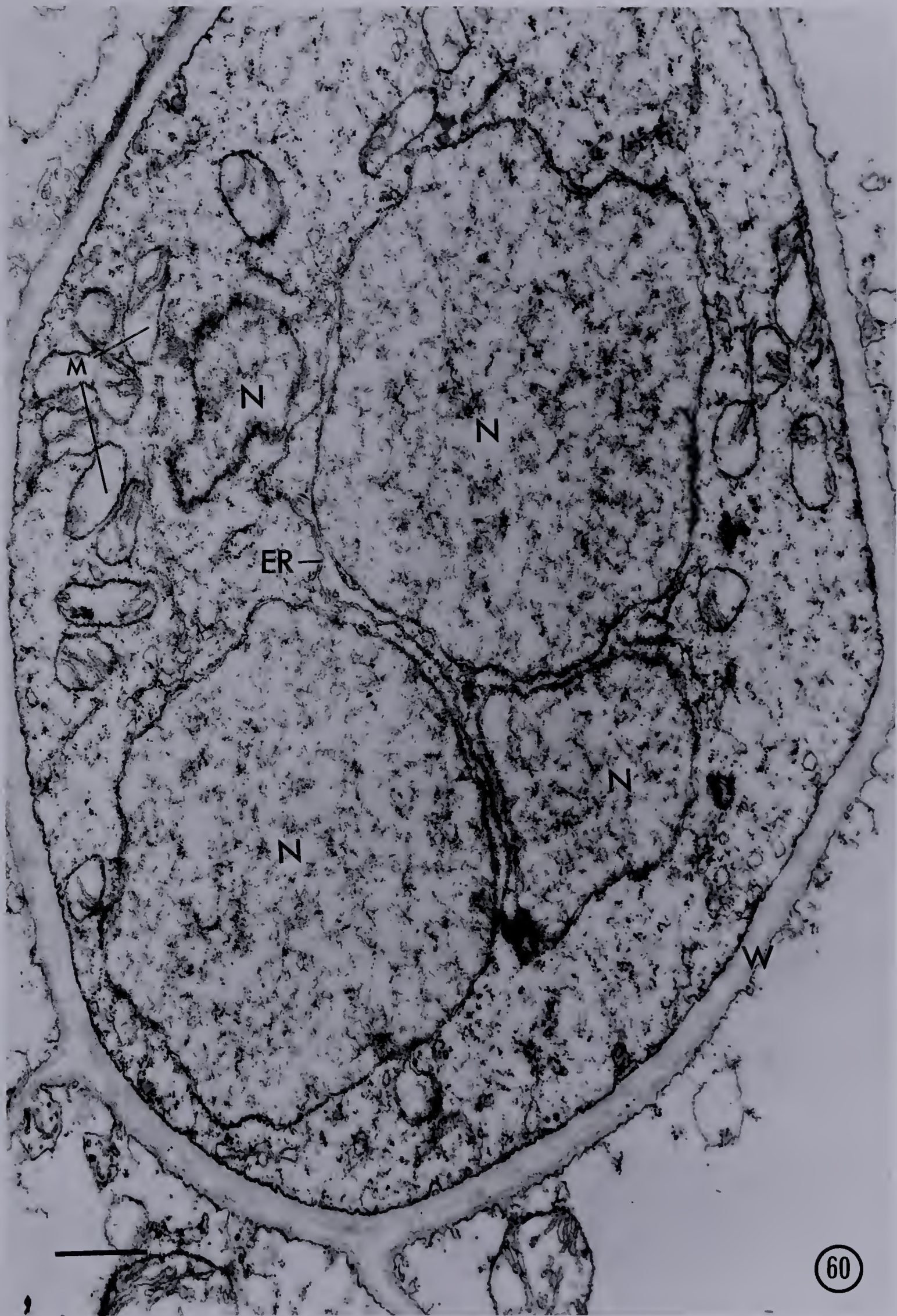
Fig. 61.   Basidium of _Coprinus lagopus_ at tetrad stage.

N, nucleus; Nu, nucleolus; cf, chromatin

fibrils; V, vacuole; R, ribosomes; M,

mitochondria (arrows mark mitochondrial

particles).  Magnification approximately

29,400 X.

61
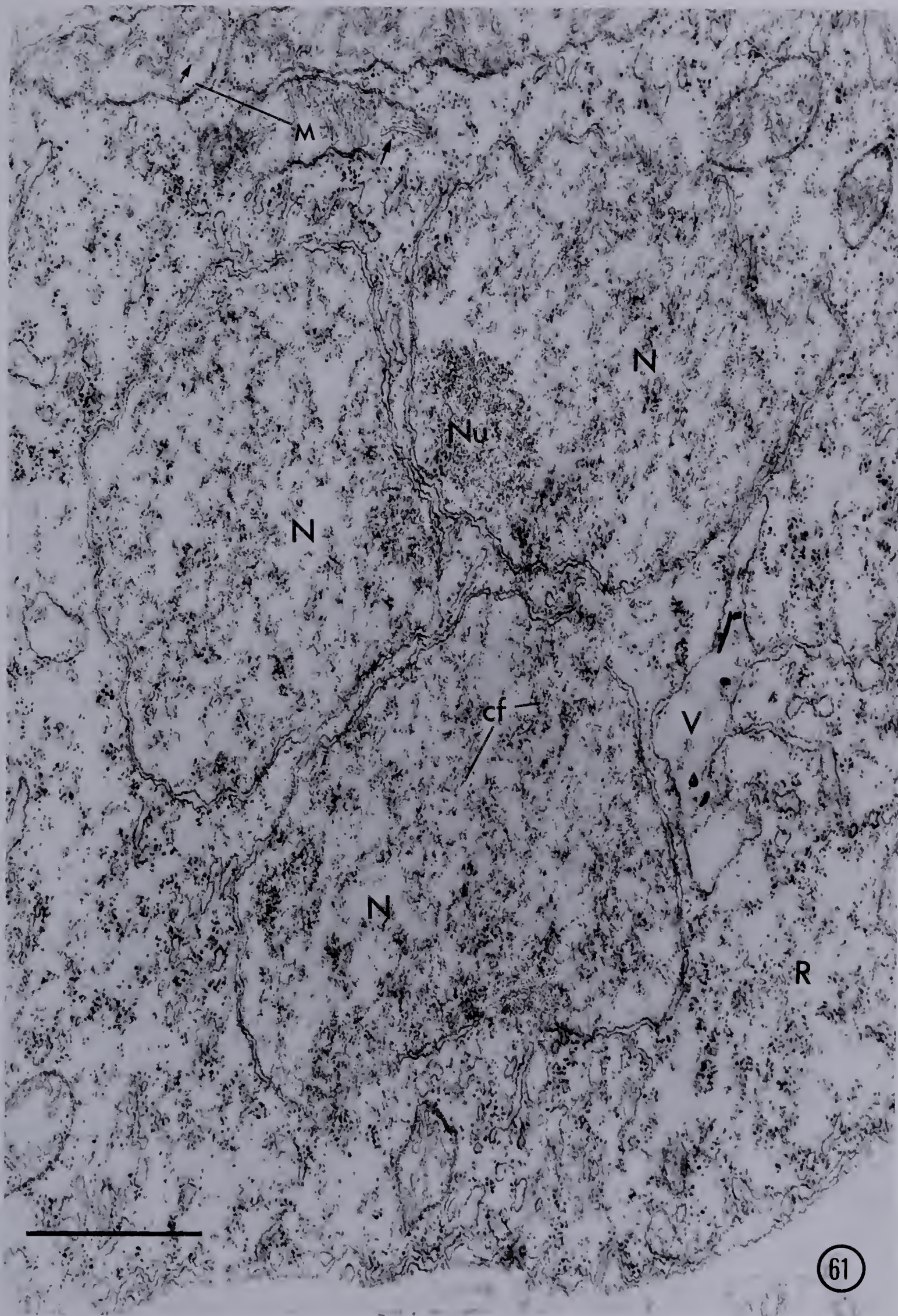
spindle is discernible. A similar configuration was observed
in <u>Cyathus stercoreus</u> (Lu, unpublished). These observations
indicate the existence of a structural spindle, which is formed
between two centrioles before the orientation of chromosomes at
the equatorial plate.

4. <u>Basidiospore Production</u>

The process of spore formation can be observed in detail
by light microscopy. After meiosis, four protuberances are pro-
duced from the apex of the basidium (Fig. 62). These represent
an early developmental stage in the formation of sterigmata on
which basidiospores are borne. Light microscopy does not reveal
the internal organization and micromorphogenetic changes. These
are demonstrated herewith by means of the electron microscope.

As shown in Figs. 64 and 66, a spore is formed at the
tip of a sterigma. The spore is filled with cellular contents
especially rich in lipid globules. In contrast, the basidium
has rather few cellular organelles (Fig. 65, 66) owing to the
fact that most of the cellular contents migrate into the spores.
This is accomplished by way of the sterigmata and through the
hilum of the spore (Fig. 67). When a spore is fully grown, a
cross-wall is laid down (Fig. 66 arrow), between the spore and
the sterigma. Subsequently, the spore wall thickens and a layer
of osmiophilic substance is laid down on its external surface
(Fig. 67).

Fig. 62.   Basidium of <u>Coprinus</u> <u>lagopus</u> after meiosis

is complete, showing sterigma formation (Stg);

M, mitochondira; ER, endoplasmic reticulum;

N, nucleus; Lo, lomasome.   Magnification

approximately 18,000 X.


Fig. 63.   A portion of the hymenium showing a basidium

at the spore-producing stage and its sur-

rounding sterile cells.   Bas, basidium; V,

vacuole; Gr, granules.   Magnification approx-

imately 12,000 X.
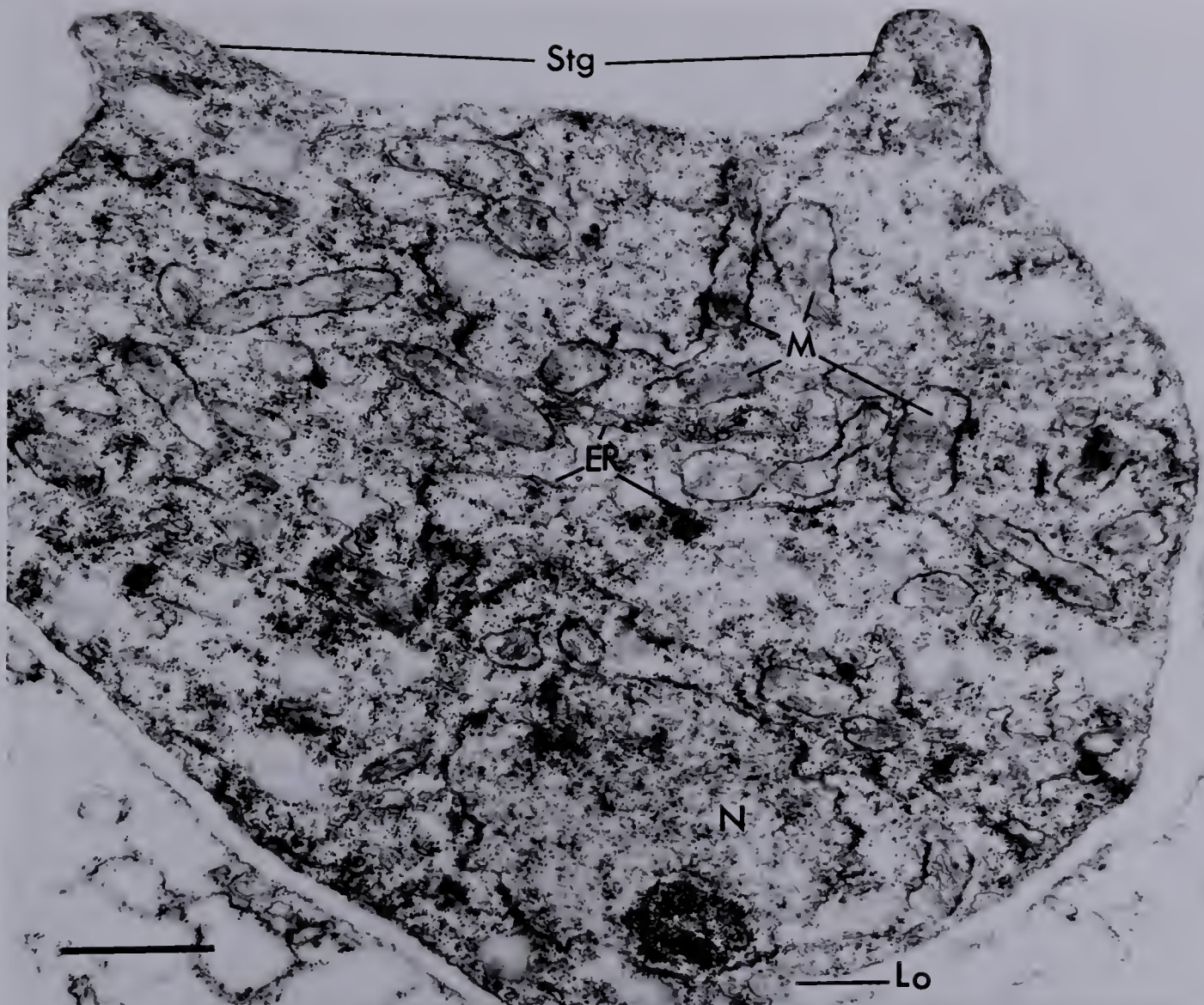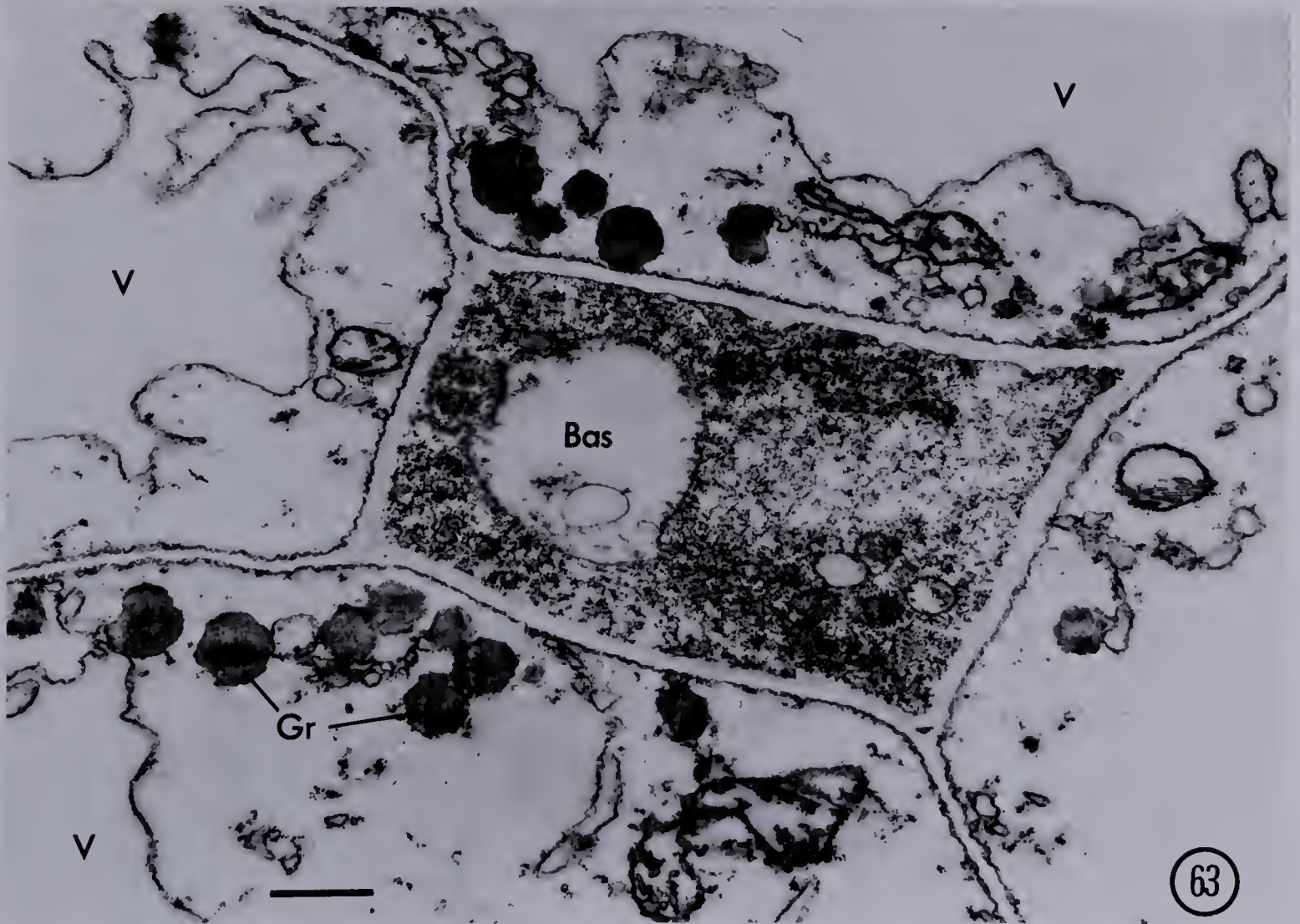
Fig. 64. Basidiospore formation showing aggregation
of fat globules (f). Magnification approx-
imately 12,000 X.


Fig. 65. Basidium from which the basidiospore, shown
in Fig. 64, is formed. Stg, sterigmata;
Er, endoplasmic reticulum, expanding to form
vacuole; V, vacuole. Magnification approx-
imately 10,000 X.

64



Stg

ER

V

65
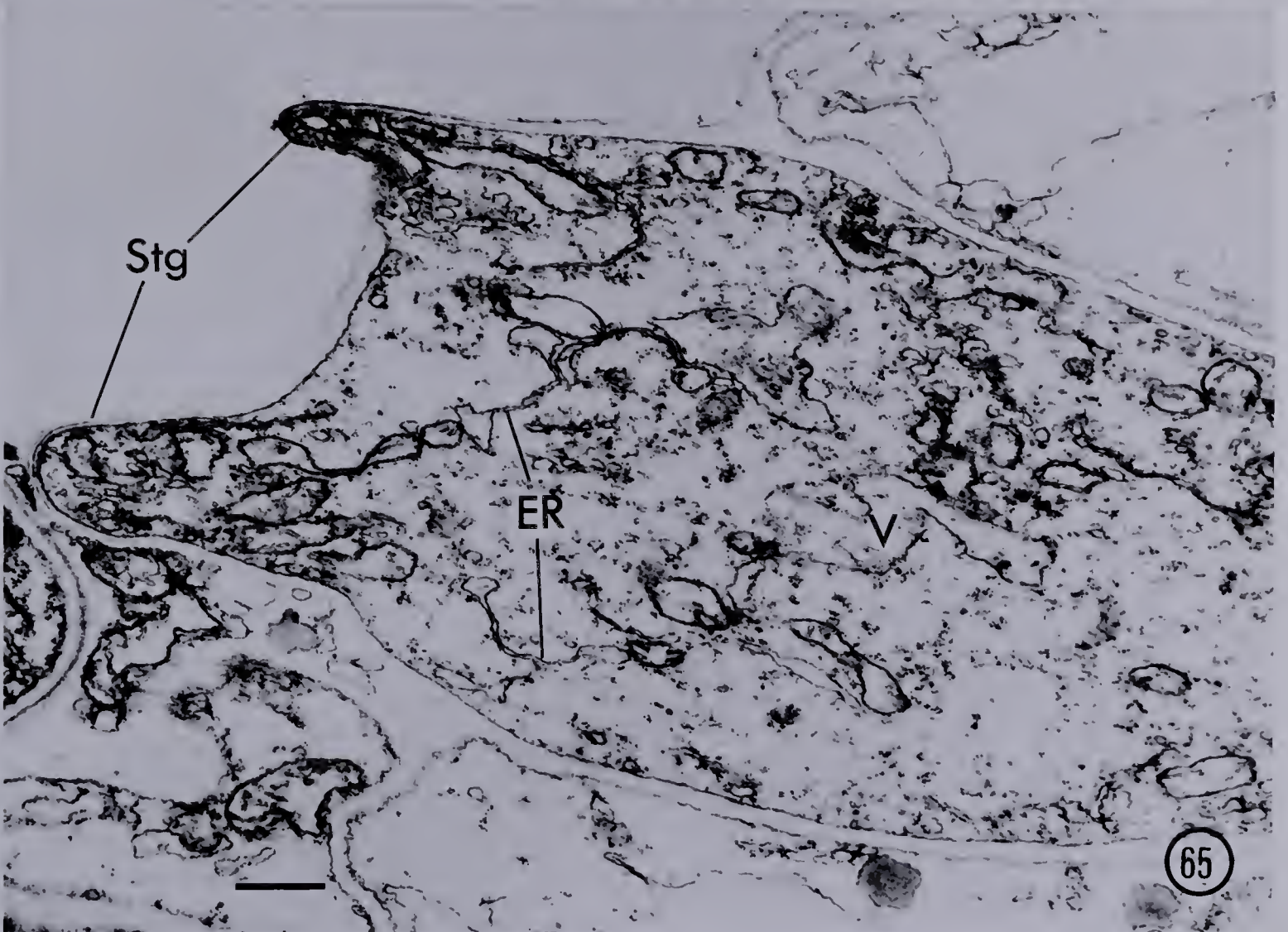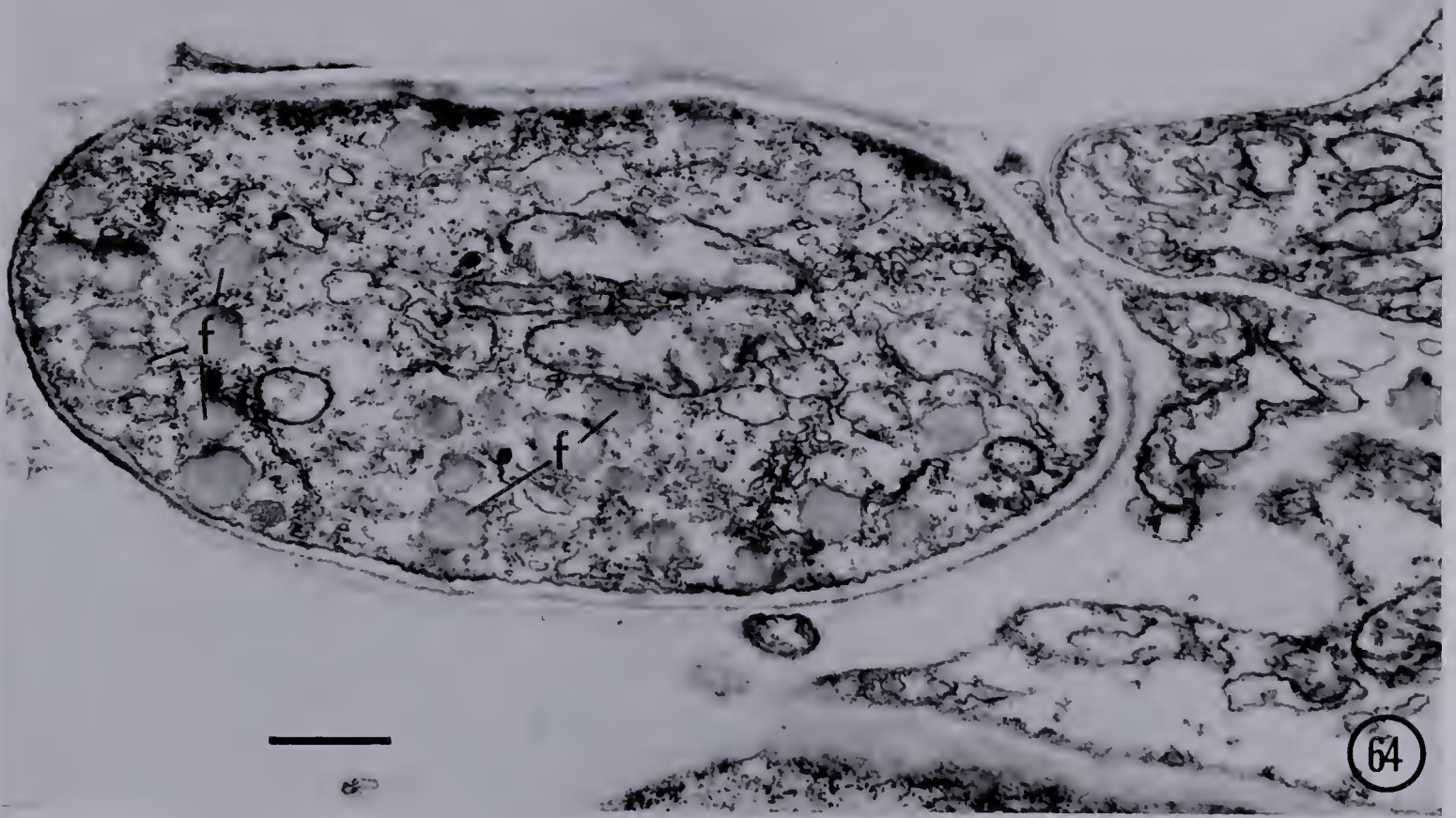
Fig. 66.   Basidiospore of <u>Coprinus</u> <u>lagopus</u>, showing
           aggregates of fat globules (f); arrow marks
           the cross wall between the basidiospore and
           the sterigma.  Magnification approximately
           12,700 X.


Fig. 67.   Mature basidiospore of <u>Coprinus</u> <u>lagopus</u>,
           showing hilum (H) and the thick osmiophilic
           wall material external to the chitin wall
           (W).  Magnification approximately 17,300 X.
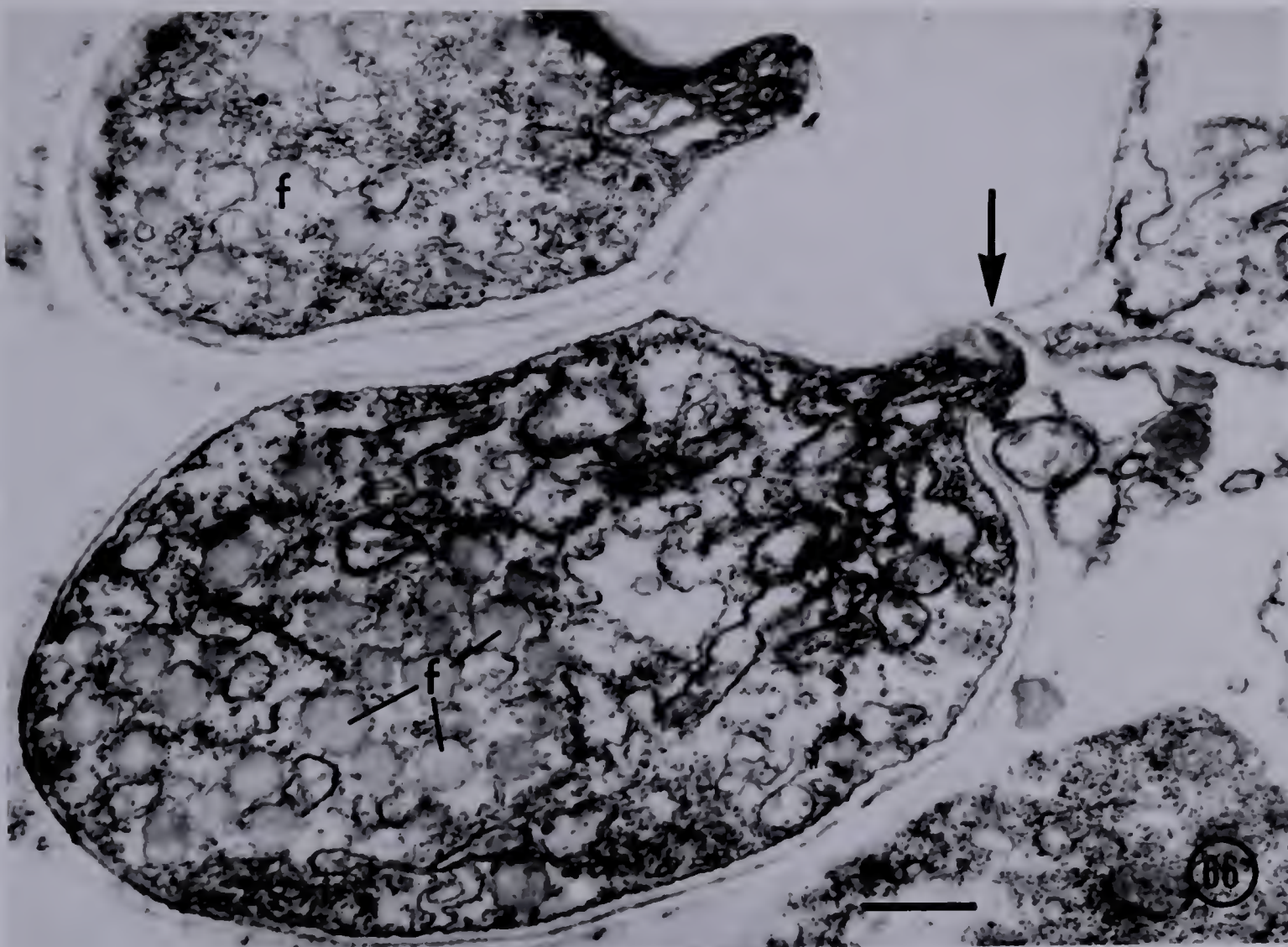
No apparent aggregation of lipid globules has been observed at any period during the course of basidial development previous to the spore-producing stage. This sudden micromorphogenetic change involving the accumulating of lipid globules may be taken as indicative of a metabolic shift in favor of lipid condensation. It is thus deduced that the lipid globules are stored foods which provide a good supply of energy for the spores; this undoubtedly is necessary for their survival.

The Fine Structural Organization of the Nuclei and Chromosomes of Coprinus

1. The Internal Organization of the Nuclei

The nuclear membrane consists of two electron-dense layers each about 90 Å thick. The membrane is porous. In Fig. 68 an area of an oblique section of the nuclear membrane is seen in surface view and the nuclear pores (or the annuli) can be seen. These measure about 60 - 90 mμ in diameter. The magnitude of the pores is about the same as that of the pores of Exidia nucleata (Wells, 1964a). Around the annulus, there are microtubules. These are perpendicular to the membrane. Microtubules have not been described for nuclear pores of fungal material, although they have been reported in the annuli of the nuclear membrane of animal cells (Bernhard, 1959; Kessel, 1965; Afzelius, 1955). In the annulus proper, there is a very fine network which exhibits a discernible pattern of organization. There appear to be two compartments in the center, connected to the surrounding microtubules by nine very fine fibrils (Fig.

Fig. 68.   Nucleus of a sterile cell of Coprinus

lagopus, showing surface view of the nuc-

lear membrane (NM) and its pores (Np).

Note that the pore is made up of micro-

tubules, probably in 9-2 arrangement (see

text).   Magnification approximately 100,000

X.


Fig. 69.   Basidium of Coprinus lagopus at tetrad

stage, showing a nucleus (N) and its assoc-

iated centriole (c); Nu, nucleolus; M,

mitochondria; R, ribosomes; ig, intracister-

nal granules.   Magnification approximately

25,000 X.
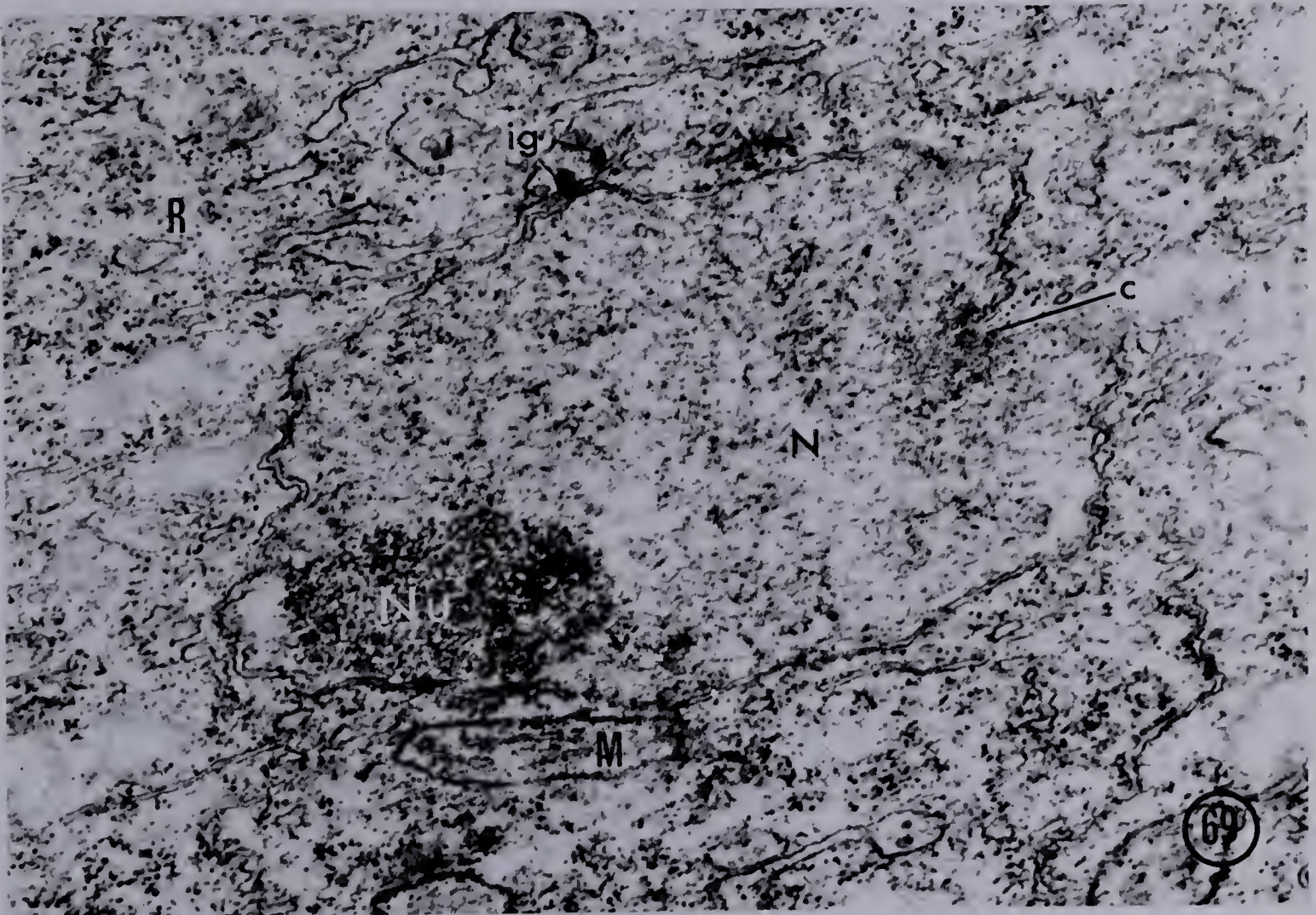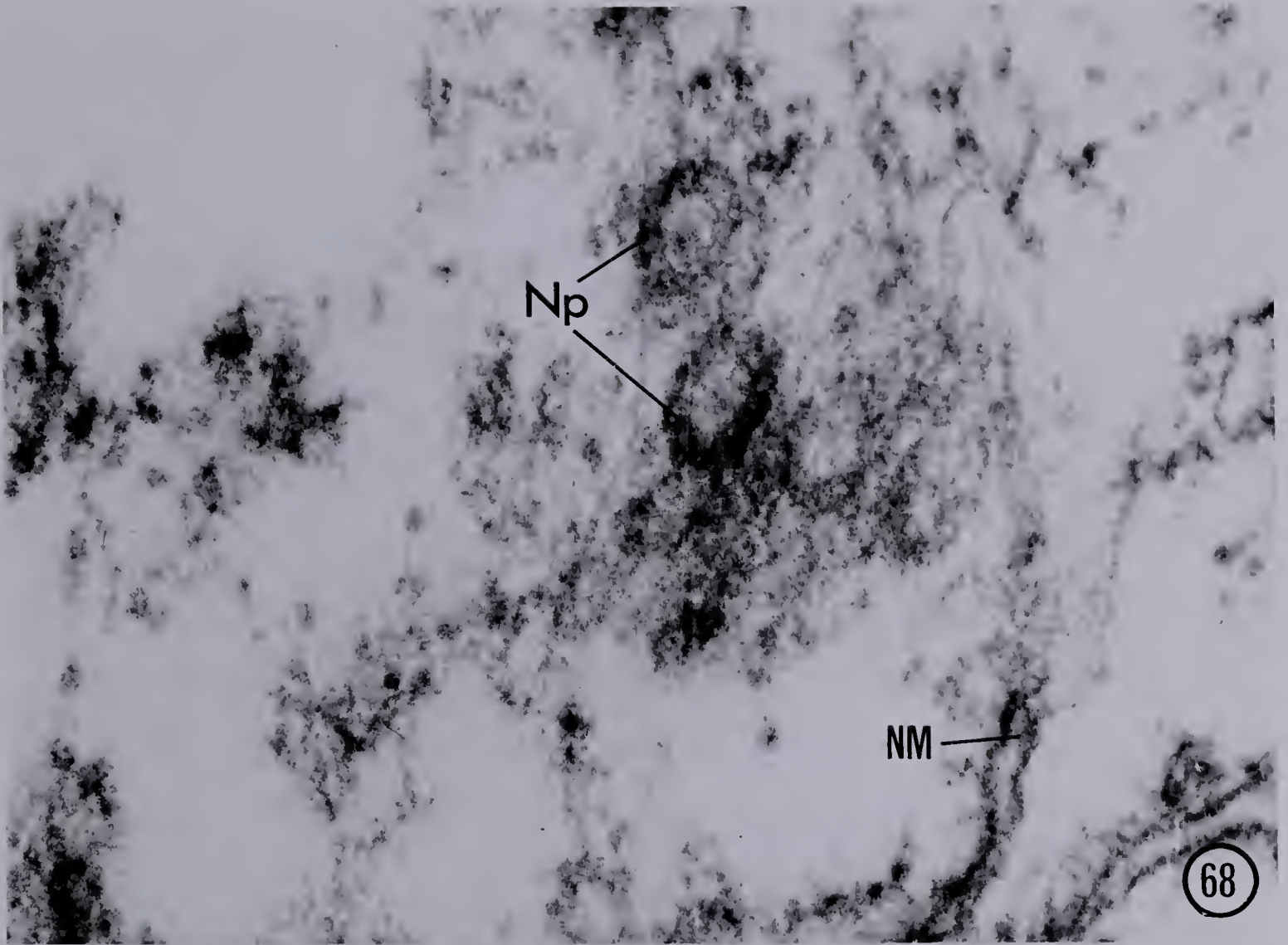
68). The presence of these nine fibrillar pillars suggests that there may also be nine surrounding microtubules.

Inside the nucleus, there are electron-dense fibrils about 100 - 150 Å thick (Fig. 56, 70, 71, 72, 73); these are believed to be the basic components of chromosomes. Careful examination in favorable areas reveals that these 100-Å fibrils represent the result of regular coiling of a single 20 - 30 Å subfibril which is thought probably to be the DNA-protein complex (Fig. 56, small arrows). The fibrils are embedded in the nuclear sap, which exhibits a fine, even, electron-sparse texture similar to that of the cytoplasmic matrix.

In a diploid nucleus, chromatin fibrils have a bumpy appearance (Fig. 71, arrow) quite in agreement with the fibrils revealed by whole-mount electron microscopy (DuPraw, 1964; Wolfe, 1965). The bumpiness suggests that the chromatin fibrils are twisted.

In Fig. 72, a nucleolus is observed near the nuclear membrane, It is characterized by its very electron-dense, granular strucutre. Inside the nucleolus, fibrillar structure is evident.

The fine structure of interphase nuclei in cystidia has unique characteristics (Fig. 73). These nuclei, as far as is known, are in a steady state and no further nuclear division will later take place. Under a light microscope, they resemble

Fig. 70.  Nucleus (N) of _Coprinus_ _lagopus_ at meiotic

prophase showing chromosome (Ch) embedded

in clear nuclear sap; M, mitochondria; R,

ribosomes; V, vacuole.  Magnification approx-

imately 47,500 X.
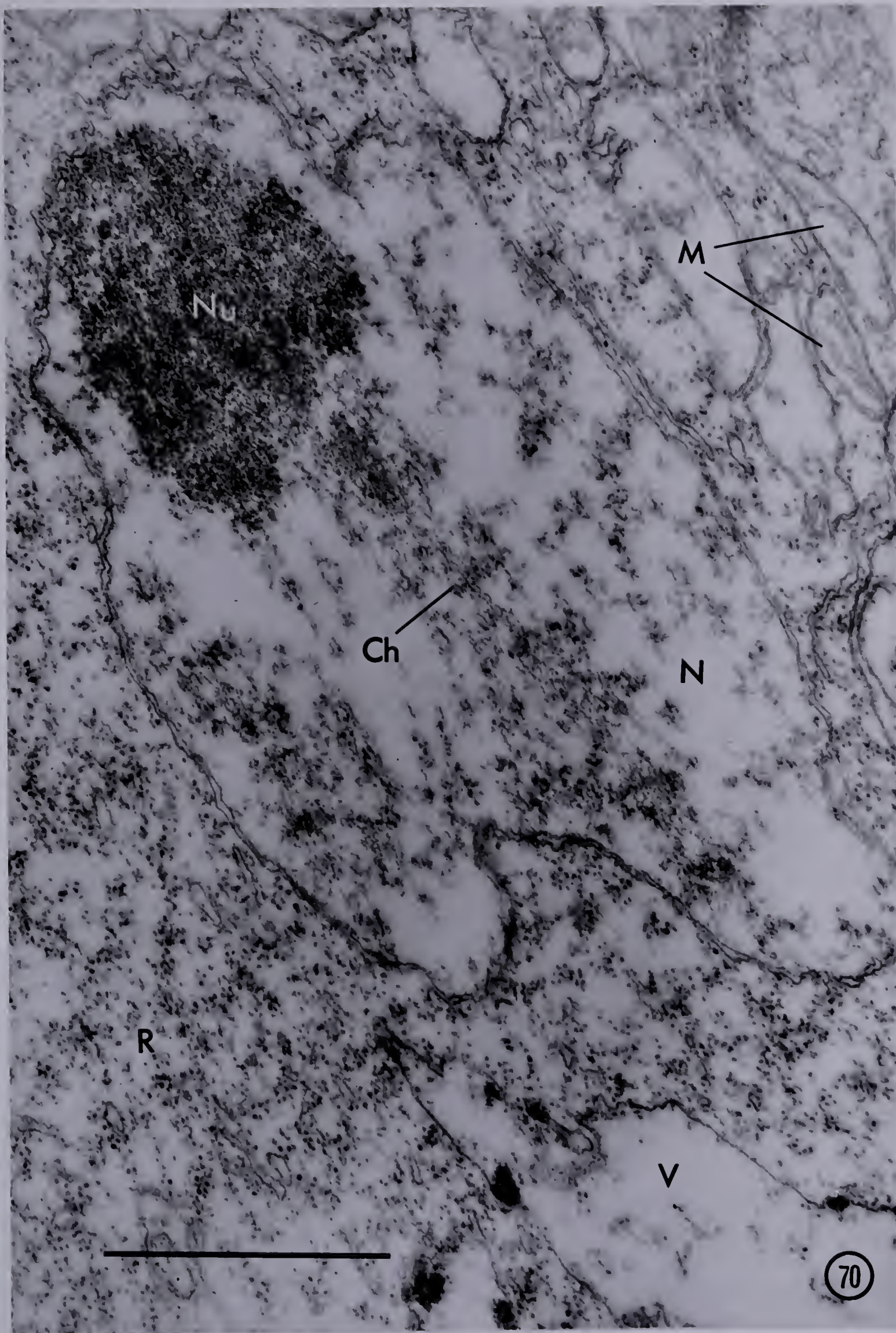
Fig. 71.  Nucleus (N) of _Coprinus lagopus_ at meiotic

prophase.  Chromatin fibrils (cf) exhibit

bumpiness (large arrow); R, ribosomes.

Magnification approximately 60,000 X.


Fig. 72.  Nucleus of _Coprinus lagopus_ at meiotic

prophase (Synapsis) showing a prominent

nucleolus (Nu). Note the granules - the

size of ribosomes, in the nucleolus; Syn,

synaptinemal complex; ba, the synaptic

center (or bivalent axis); cf, chromatin

fibrils.  Magnification approximately

60,000 X.
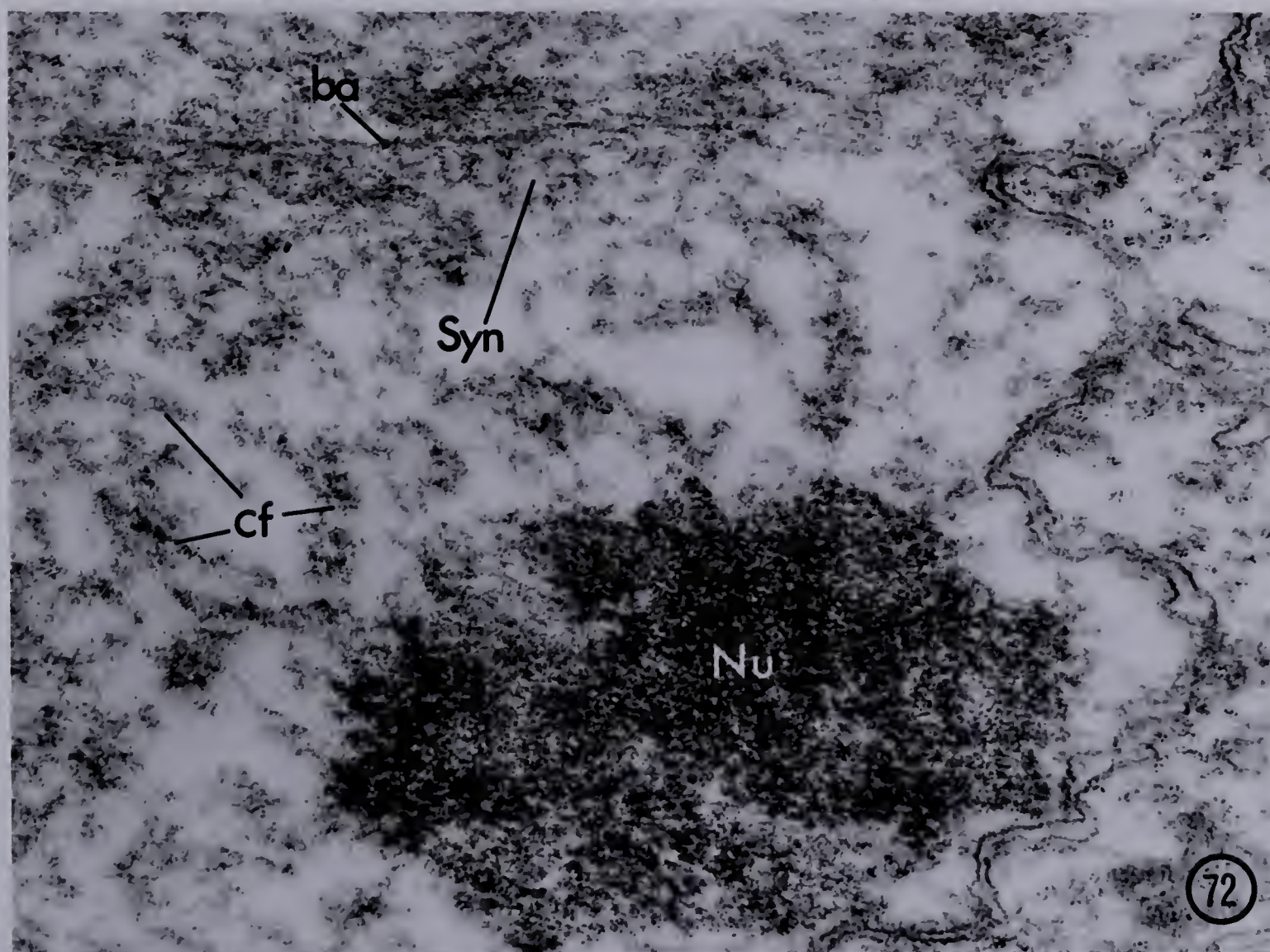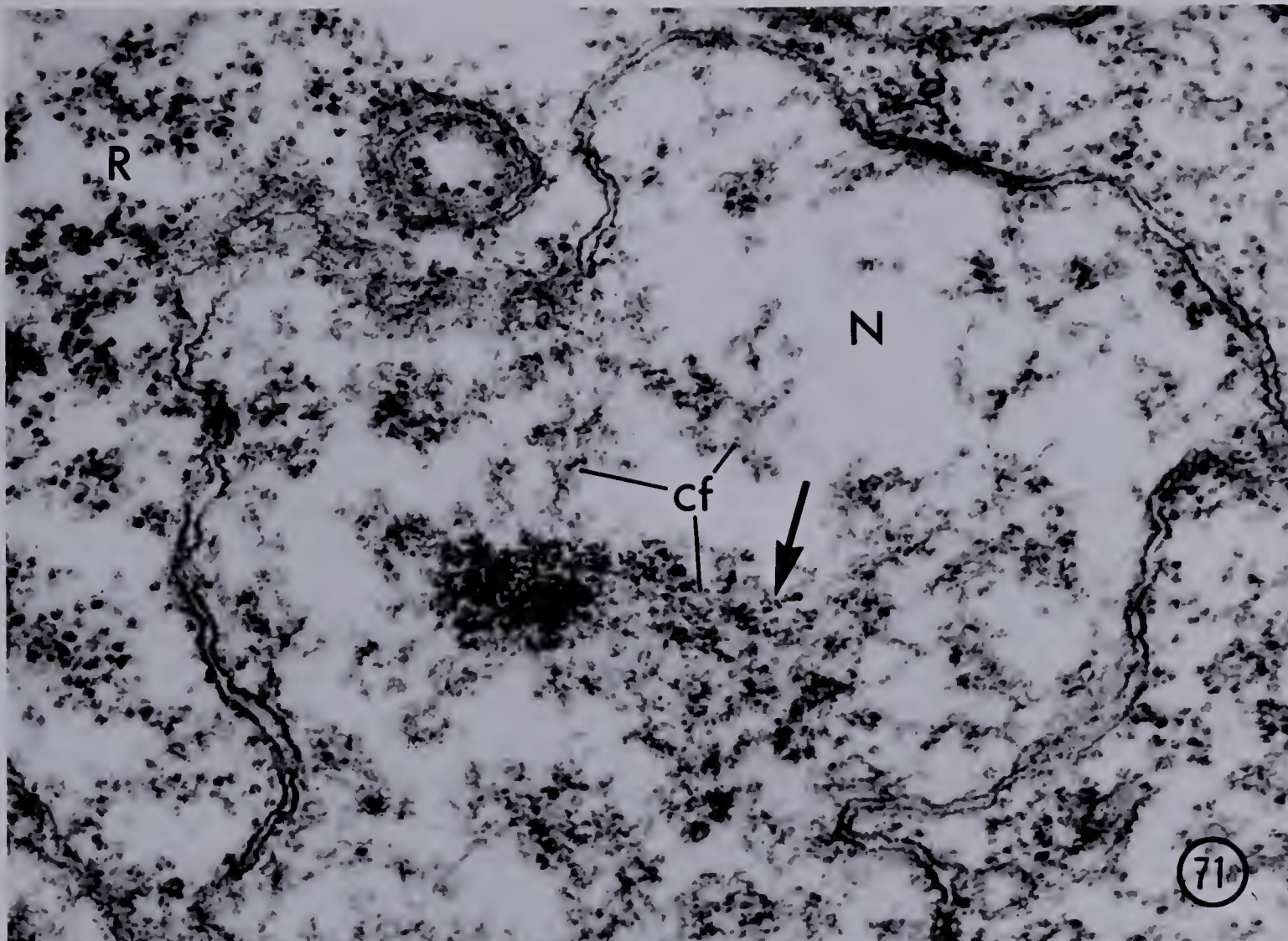
Fig. 73.   Nucleus (N) of the cystidium of _Coprinus_

_lagopus_, showing chromatin network.  Chrom-

osomes (Ch) are identifiable.  Note that

chromosomes are made up of foldings of fibrils

(large arrow).  The small arrow marks a

chromosome end associated with the inner

nuclear membrane where two fibrils are seen.

Magnification approximately 40,500 X.

N

Ch

73
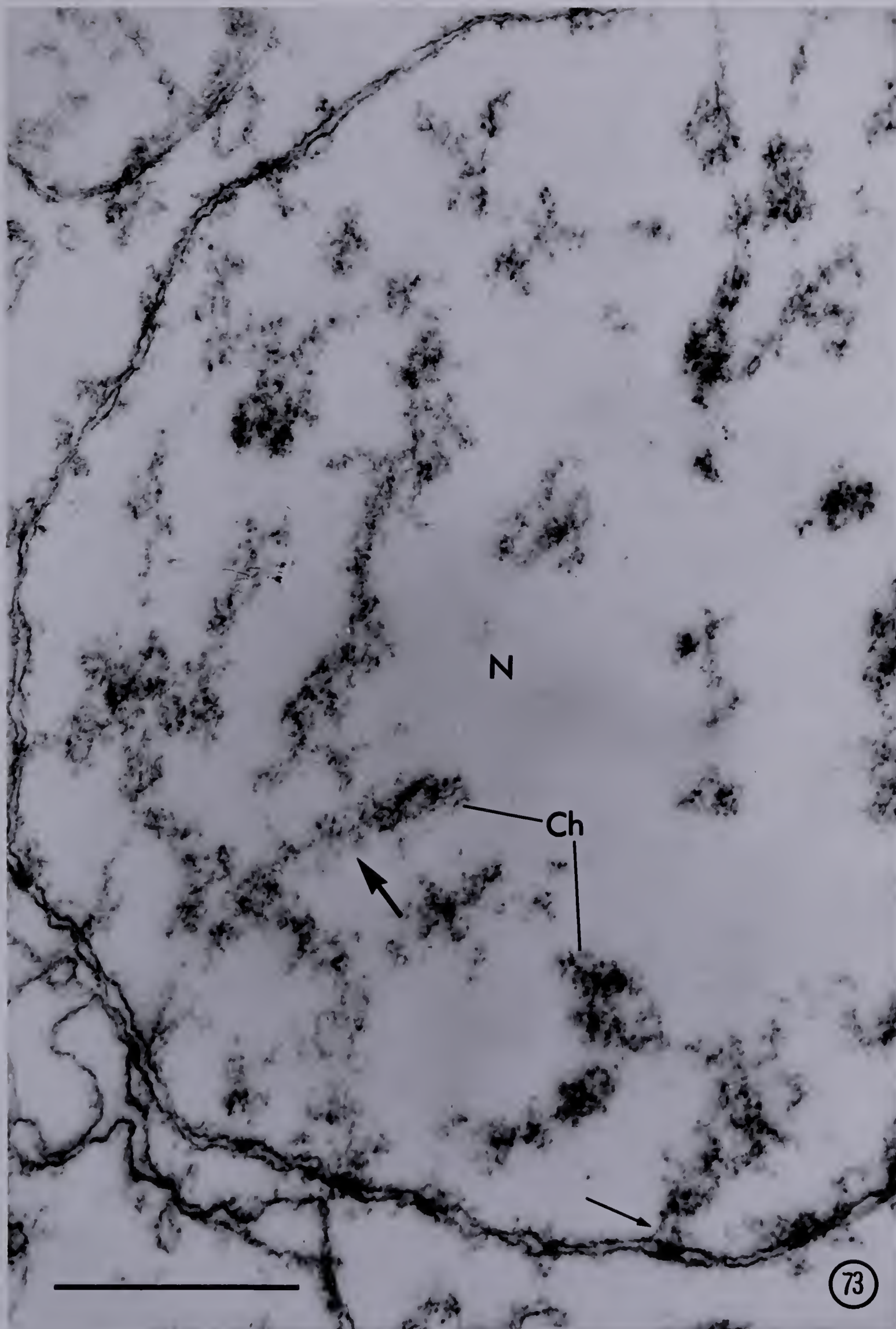
interphase nuclei of the mycelium in size and shape. The chroma-
tin network can be easily seen. In favorable sections, the same
kind of network is apparent and the continuity of chromosome is
preserved (Fig. 73).

As shown in Fig. 73, the chromosomes appear to consist
only of fibrils which become entangled to form a chromosome approx-
imately 0.1 - 0.3 μ thick. The large arrow in Fig. 73 marks a
chromosome which appears to consist of folded fibrils. Although
the pattern of organization of these fibrils cannot be discerned,
one gains the impression that the chromosomes consist of one or
two single DNA-protein fibrils folding laterally to form a chrom-
osome. Of particular interest is the chromosome seen at the
lower right of Fig. 73, where the chromosome end contacts the
inner nuclear membrane. It appears that there are two strands
of 100-Å fibrils. This indicates that a chromosome of Coprinus
consists of two strands. Whether or not these two strands re-
present chromatids is not certain (see discussion).

2. The Fine Structural Organization of Meiotic Chromosomes

It has been established that the organization of fungal
chromosomes as revealed by light microscopy resembles that of
other eukaryotes (McClintock, 1945; Singleton, 1953; Knox-Davies
and Dickson, 1960; Lu and Brodie, 1964; Lu, 1964a, 1964b). Com-
parative observations of pachytene and diplotene chromosomes of
Coprinus lagopus by means of light microscopy and electron micro-
scopy provide further evidence that the resemblance extends to
the fine structural organization of meiotic chromosomes.

Fig. 74.  Nucleus (N) of _Coprinus_ _lagopus_, showing:
Syn, a synaptinemal complex; ca, homologous-
chromosome axes; ba, synaptic center (or
bivalent axis); pf, chromosome puff (note in
symmetrical pair); M, mitochondria associated
with the nucleus.  The small arrows mark the
lateral loop ends which line up at the pair-
ing surface to form the synaptic center.
Magnification approximately 114,000 X.

M

pf

Syn →

ba

pf

ca

ca

N

0.1 μ

74
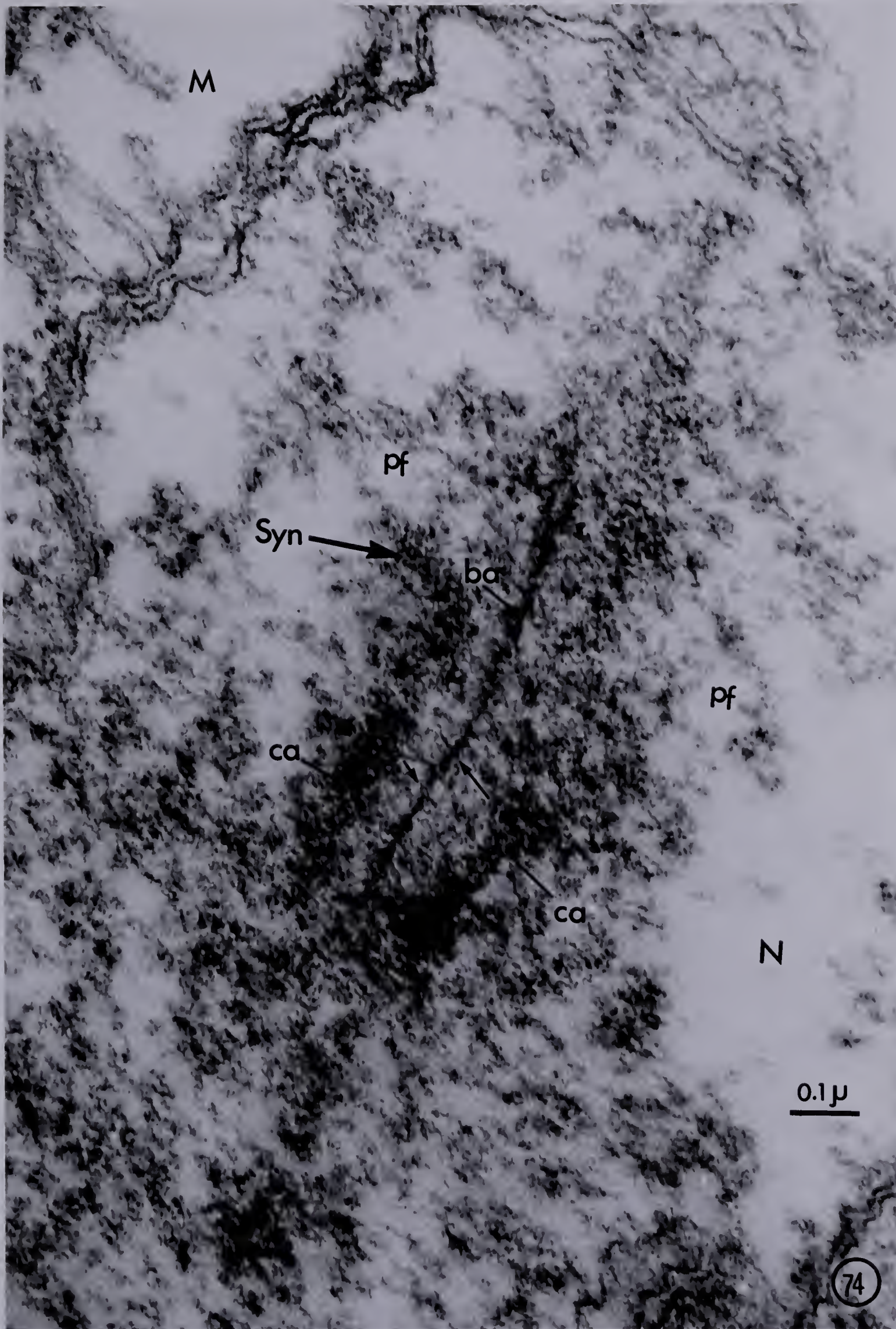
## The Fine Structure of Pachytene Chromosomes

As described earlier, a bivalent chromosome, when
sectioned in a frontal plane passing through the axes of both
homologues as indicated in Diagram 1, AB, and examined under the
electron microscope may be expected to exhibit a tripartite struc-
ture.  This structure is comparable to the synaptinemal complex
of higher organisms (Moses and Coleman, 1964; Moses, 1958, 1960;
Coleman and Moses, 1964; Ris, 1961; Nebel and Coulon, 1962a;
Meyer, 1960).

In Fig. 74 is shown a tripartite synaptinemal complex
which stands out against the somewhat clear background of the
nuclear sap.  This complex may be slightly twisted in the section
plane, which may account for the lack of perfect symmetry.  Never-
theless, it is apparent that the two homologous chromosomes are
mirror images of one another in the area where a pair of chromo-
some "puffs" (pf) is formed.  Similar complexes are shown in
Fig. 75: the one at the left (1) is not perfectly symmetrical,
suggesting that the two homologues are twisted; the other, at
the right (2), illustrates the direct attachment of the two
homologous chromosomes to the inner nuclear membrane (Fig. 75,
large arrow).

The two lateral elements are interpreted as the axes
of the two homologues.  They are about 500 - 800 $\overset{\circ}{A}$ thick and are
symmetrically spaced from the central element about 150 $\overset{\circ}{A}$ thick.
The central element is interpreted as being the pairing surface
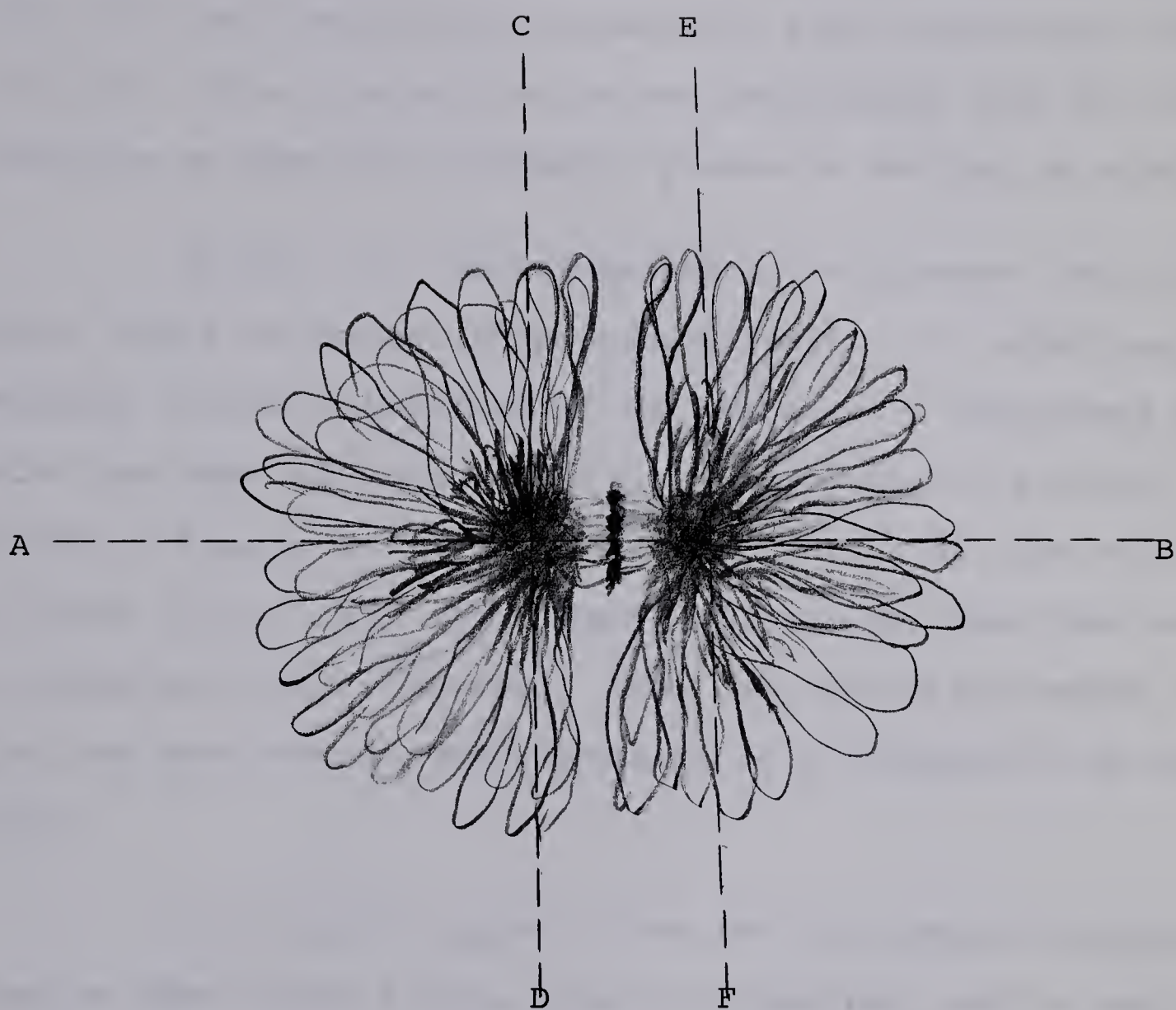
Diagram 1.  A diagrammatic drawing of a view of the cross section through a bivalent (the synaptinemal complex as shown in Figs. 75 and 77) showing two homologous chromosomes and the synaptic center.  AB represents the frontal plane of the longitudinal section through the axes of both homologues; CD or EF represents the sagittal plane through the axis of a single homologue.

(or synaptic center) where two homologues meet.

The belief that the lateral elements are the homologous-chromosome axes, is derived from a direct comparison made between the synaptinemal complex as revealed by electron microscopy (Fig. 74) and bivalents as revealed by light microscopy (Figs. 39 - 41).  This view may be further established when the fine structure as seen from different planes of section is studied.

In Fig. 75, apart from the two structures described above, there is another chromosome structure (3) which suggests strongly a view of the axis of one member of a homologous pair which has been cut through the CD or EF plane (or sagittal plane) of Diagram 1.  (A higher magnification of this structure is shown in Fig. 76).  The other homologue may have been above or below the section studied.  This interpretation agrees with what has been seen in cross sections of a bivalent to be described later.

It is quite apparent that the homologous-chromosome axes as seen in the frontal plane (AB section) and in sagittal plane (CD or EF section) are identical in nature (Fig. 75, also compare Figs. 74 and 76).  Thus these two views allow a two-dimentional reconstruction of the axial structure from which it appears that the axial element is a structurally homogeneous, slightly flattened cylinder.  This interpretation is borne out by evidence presented later by the cross section of a bivalent.

Fig. 75. Nucleus (N) of _Coprinus lagopus_, showing 1 and
2, a frontal plane of two synaptinemal complexes,
Syn; 3, a sagittal plane of a homologous
chromosome; 4, a transverse section of a
chromosome revealing radiating lateral loops
(or bristles) from the chromosome axis, ca;
5, a transverse section of a synaptinemal
complex revealing two homologous-chromosome
axes, ca. Magnification approximately 37,000
X.
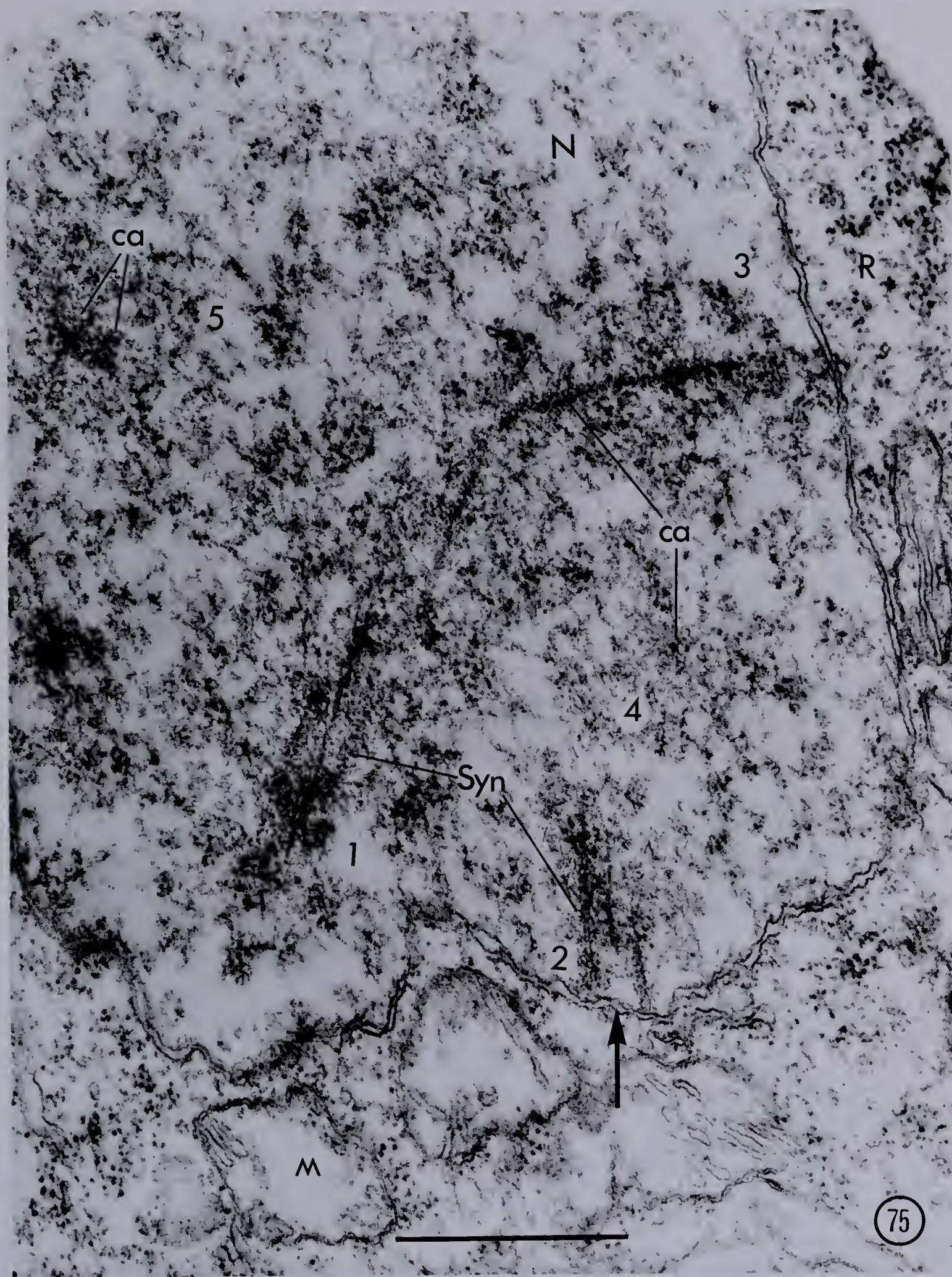
Most of the chromatin microfibrils are arranged around the homologous-chromosome axes. This can be seen in frontal section (Fig. 74), in sagittal section (Fig. 76), as well as in cross section (Figs. 76, 77). The bulk of fibrils may represent lateral projections, presumably loops, from the chromosome axes. Indeed, such projections are discernible where loops project toward the synaptic center (Fig. 74, small arrows, and in Fig. 54). The orientation of the lateral projections can best be seen in views of cross section (Figs. 76, 77). In Fig. 76, a view of sagittal section and a view of cross section may be compared. From the view of cross section, the lateral projections appear to have a common origin from the central region. In favorable sections, as illustrated in Fig. 77, lateral loops can be re-solved.

In cross sections of a synaptinemal complex, each homologue of a bivalent appears as a half circle or nearly so. The lateral projections are about the length of the radius of the circle, except those that project toward the synaptic center. The latter are short loops. As shown in Fig. 74, the short loops of both homologous chromosomes line up to form the synaptic center, or the pairing surface. In Figs. 77 and 79 is shown respectively a view of cross section of a complex in which the boundary of the homologous chromosomes can be clearly identified. The opposed double arrows indicate the boundary zone between the homologues. The homologous-chromosome axes of the synaptinemal complex are shown as a pair of cylinders of high electron density

Fig. 76. A higher magnification of 3 and 4 of Fig.
75 showing a saggittal section and a transverse
section of a chromosome. Note that lateral
loops (lp) radiate from the chromosome axis
(ca). Magnification approximately 78,500 X.

lp

ca

ca

N

0.1µ

lp

76
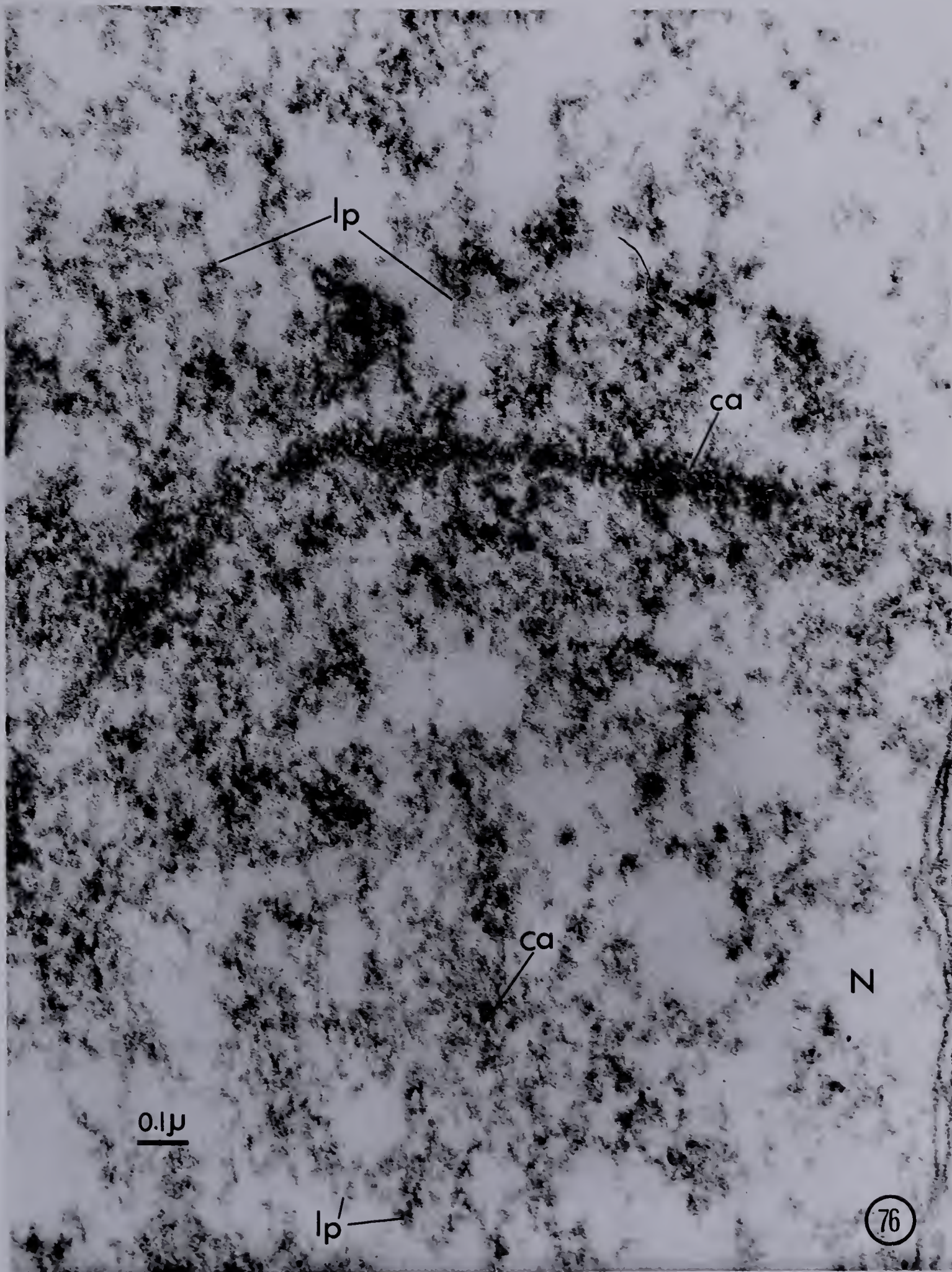
(Figs. 75(5), 79) as has been suggested above.

It is interesting to note that the homologous-chromosome axes consist of aggregates of granules, and of microfibrils arranged preponderantly at right angles (or nearly so) to the long axis of the chromosome.  It appears that the homologous-chromosome axis is made up of microfibrils like the bulk of the chromosome, but is more compact than the latter because of the aggregation.  It is here suggested that a microfibril, which runs throughout the length of a chromosome, folds laterally to form a recognizable chromosome in such a fashion that each fold passes through the vicinity of the center.  Thus when one such chromosome is sectioned either longitudinally through the axis, or transversely, it gives the pictures presented.

The synaptic center shows some interesting detail. Careful examination of this element reveals that it consists of the ends of lateral loops from the two homologous-chromosome axes (Fig. 74) small arrows) which are aligned to form the synaptic center.  This is taken to mean that the synaptic center is actually the area of effective pairing of the genetic material, as suggested by Moses and Coleman (1964).  It is suggested that the abrupt change in density at the synaptic center may be a result of the pairing of the homologous loops.  This is a reasonable assumption since the biochemical and genetic evidence is that the two homologues are identical at the macromolecular level (not considering the gene differences).

Fig. 77.    Nucleus (N) of _Coprinus lagopus_ at pachytene

stage showing a transverse section of a bivalent

chromosome (synaptinemal complex); ca, chrom-

osome axis; lp, lateral loops.  Arrows mark

the boundary of two homologous chromosomes.

Magnification approximately 52,000 X.


Fig. 78,    Nucleus (N) of _Coprinus lagopus_ at meiotic

prophase showing a chromosome (Ch).  Arrow

marks a lateral loop in which two chromatids

are identifiable.  Magnification approx-

imately 45,600 X.

Ip

Ca

R

N

77

Ch

N

78
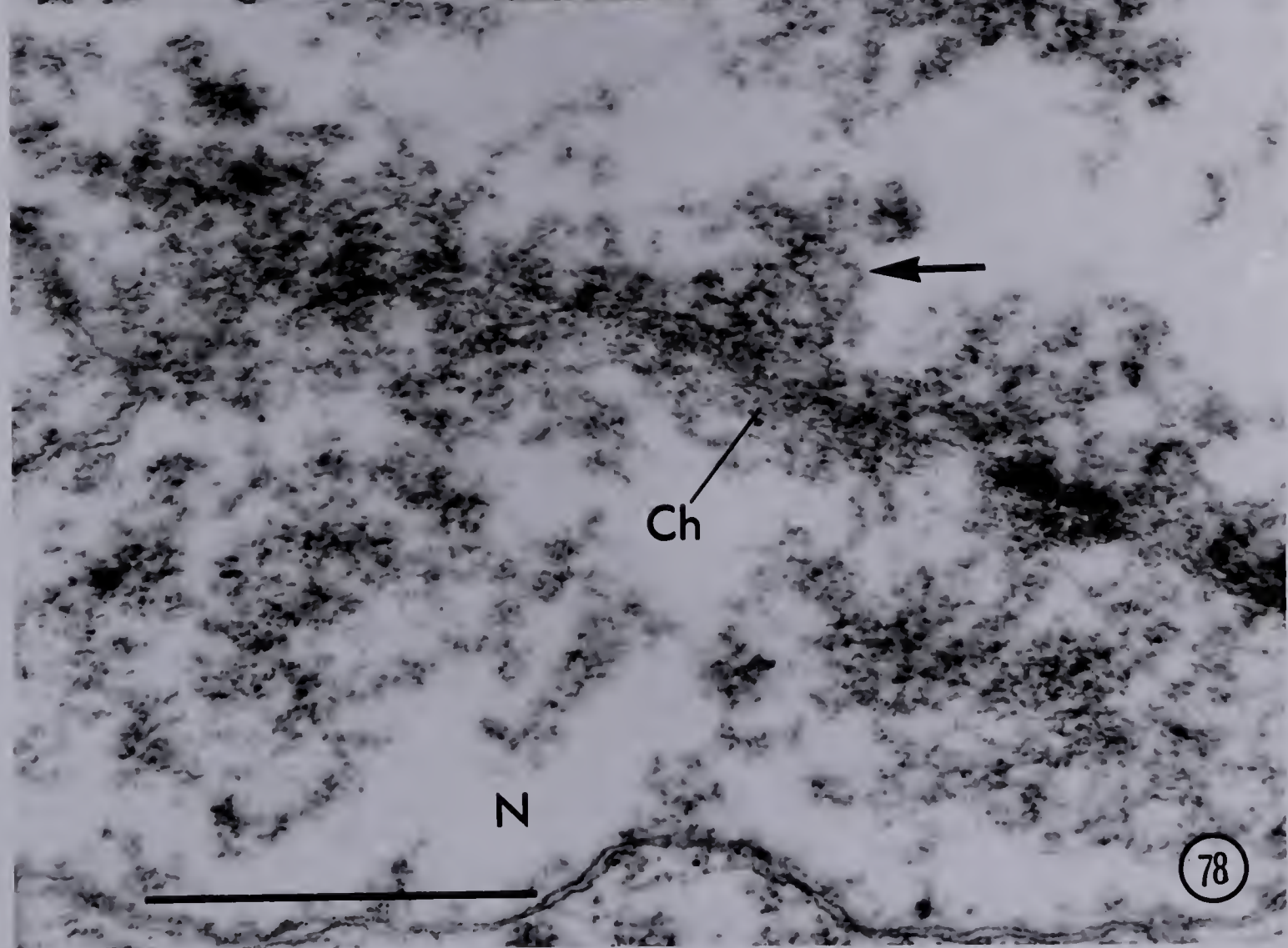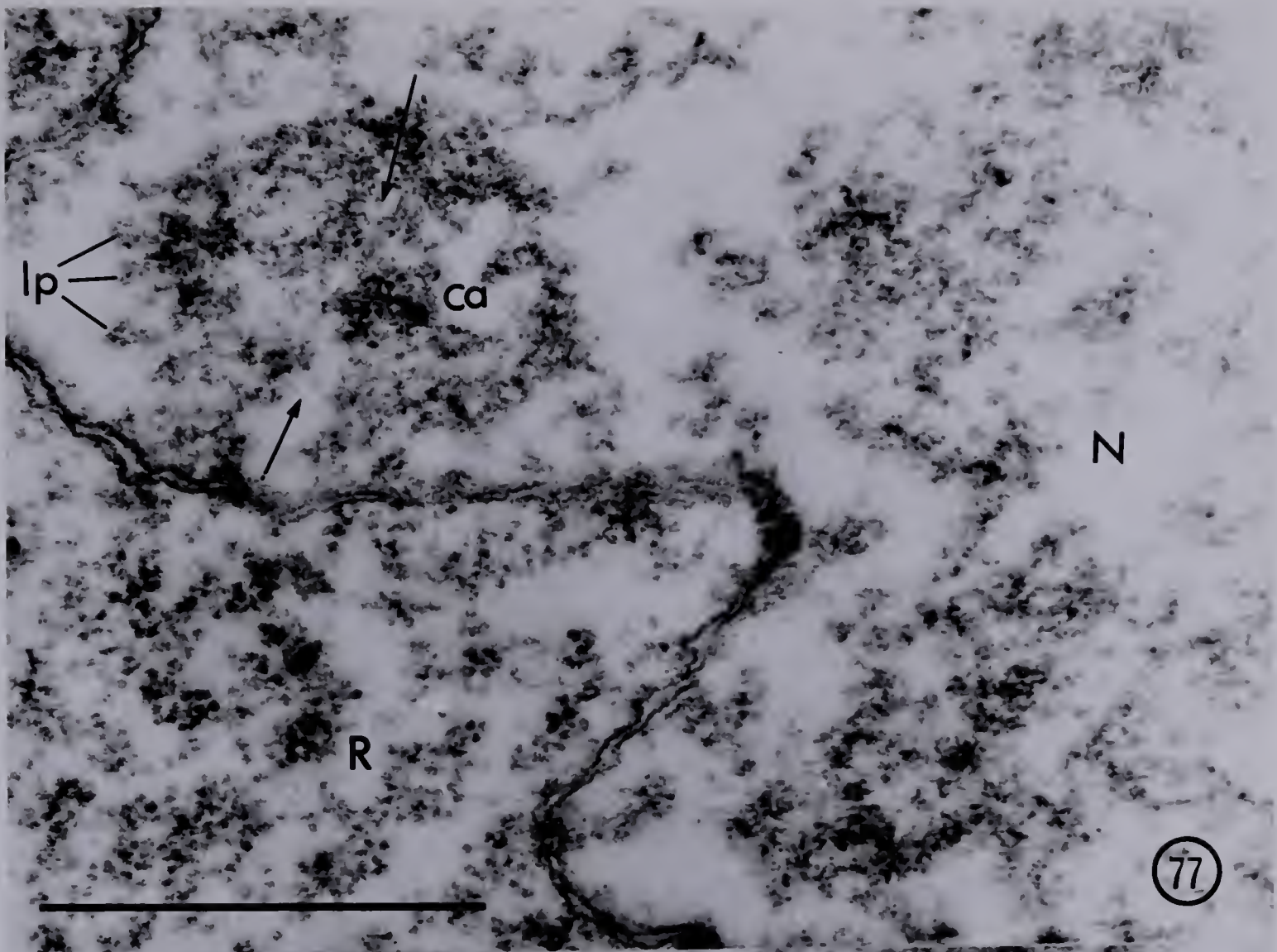
## The Fine Structure of Diplotene Chromosomes

Generally, pachytene and diplotene are two rather arbitrarily defined stages of meiosis. This is especially true for small fungus chromosomes, the identification of the division stages of which depends largely on the opinion of the workers. As can be seen from what follows, the definition of a diplotene chromosome by means of electron microscopy may be more precise than that possible with light microscopy. The two homologues of a bivalent are separated; the synaptic center is no longer present. The two chromosomes are hinged only by the chiasmata.

Fig. 57 illustrates a longitudinal section of the nucleolus-organizing chromosome at diplotene. It can be seen that the two homologues are made up of dense fibrils which contact the nucleolus. In the middle of the homologue at the right, there appears to be a constricted area, at whose center is a round body about 1000 A in diameter, which exhibits a fine texture different from the rest of the chromosome. This body is believed to be the centromere. The two homologues are separated by about 0.5 μ. Of particular interest is the area bebetween the nucleolus and the centromere. It is obvious that the two homologues join. Although the electron micrograph does not reveal much detail, it is possible that this area may represent a "chiasma" formation. Between the chiasma and the nucleolus may be seen four chromatids, each of which is individually folded. The identity of the two chromatids of a chromosome

is also shown in a lateral loop in Fig. 78 (arrow).

Fig. 79 illustrates yet another feature of a diplotene
nucleolus chromosome. Here the chromosome stands out clearly
against a background of nuclear sap because of the absence of
chromatin fibrils from other chromosomes. It can be seen that
the lateral loops project outwards from the chromosome axis and
that the latter exhibits dense patches. From the micromorpholog-
ical point of view, this pattern of organization resembles remark-
ably that of amphibians (compare with figures of Lafontaine and
Ris, 1958). Thus it is possible to assume that, at diplotene,
the chromosome of Coprinus lagopus is a miniature lampbrush type.

Fig. 80 illustrates a much condensed chromosome, pro-
bably at late diplotene. Again the chromosome stands out against
a clear background. The foldings of lateral loops may be seen.
It is obvious that this is a longitudinal section through the
axis of one of the homologous pair. The axis is still discernibly
more dense than the rest of the chromosome. It is assumed that,
as chromosome condensation proceeds, the lateral loops eventually
become as dense as the axis, which may account for the fact that
chromosomes at metaphase do not exhibit any axial differentiation.
This explanation is based on the assumption that the chromonemata
(microfibrils) fold to form a chromosome, and that the axis has
more aggregates of chromonemata per unit area than the rest of
the chromosome.

Fig. 79. Nucleus (N) of Coprinus lagopus, showing a
prominent nucleolus chromosome (Ch) in
longitudinal section, and a transverse view
of another chromosome revealing two chromo-
some axes (ca) and boundary of two homologous
(arrows). The large arrow marks a probable
chiasma where two homologues of the nucleolus
chromosome join. The small arrow at the
left marks a lateral loop. Magnification
approximately 52,000 X.

Fig. 80. Nucleus (N) of Coprinus lagopus at meiotic
prophase showing a condensed chromosome (Ch)
standing out in a clear background; the
chromosome axis is discernibly more condensed
than the lateral loops. Magnification approx-
imately 56,000 X.

Nu

N

Ch

ca

79

Nu

N

Ch

80
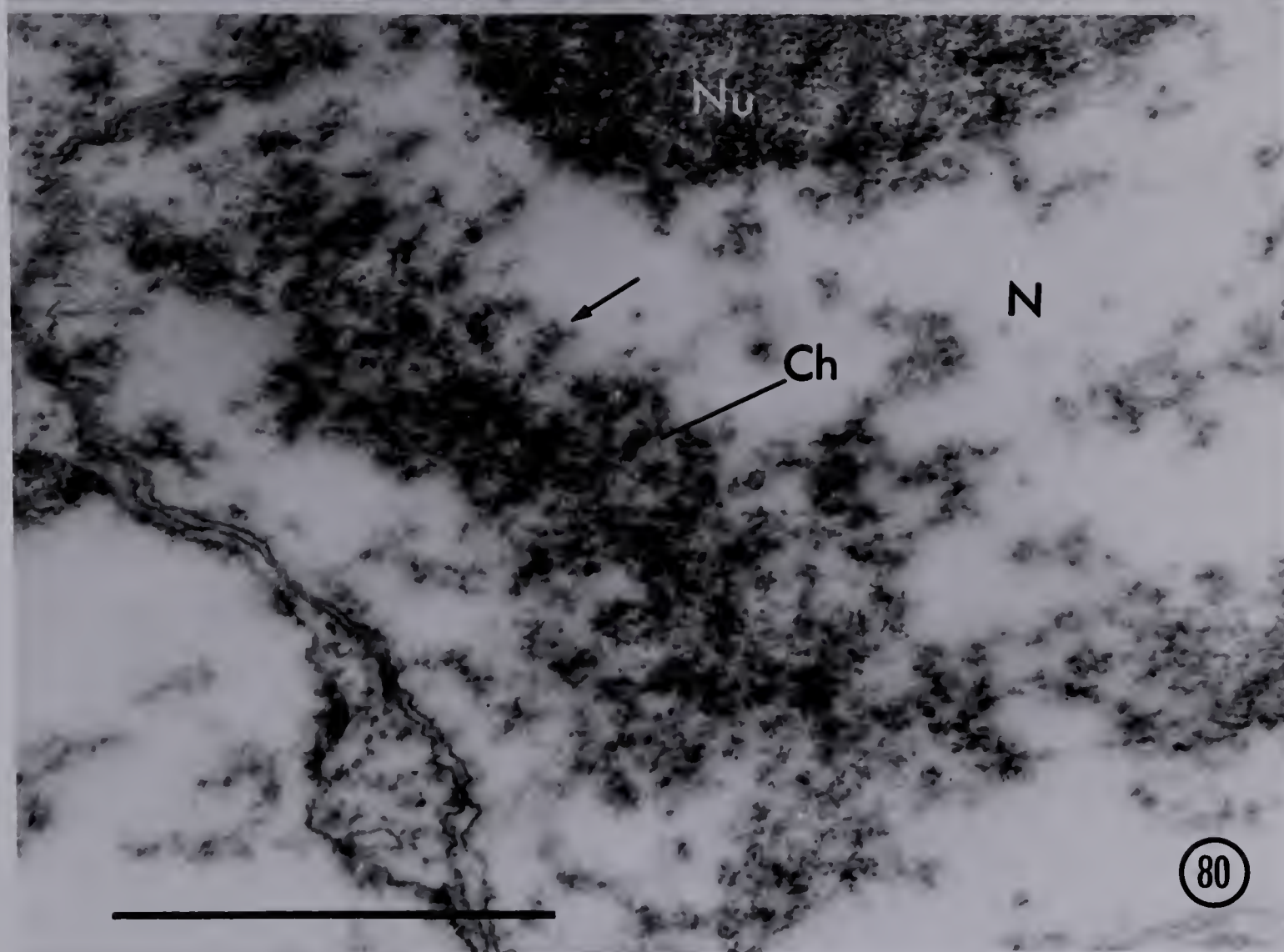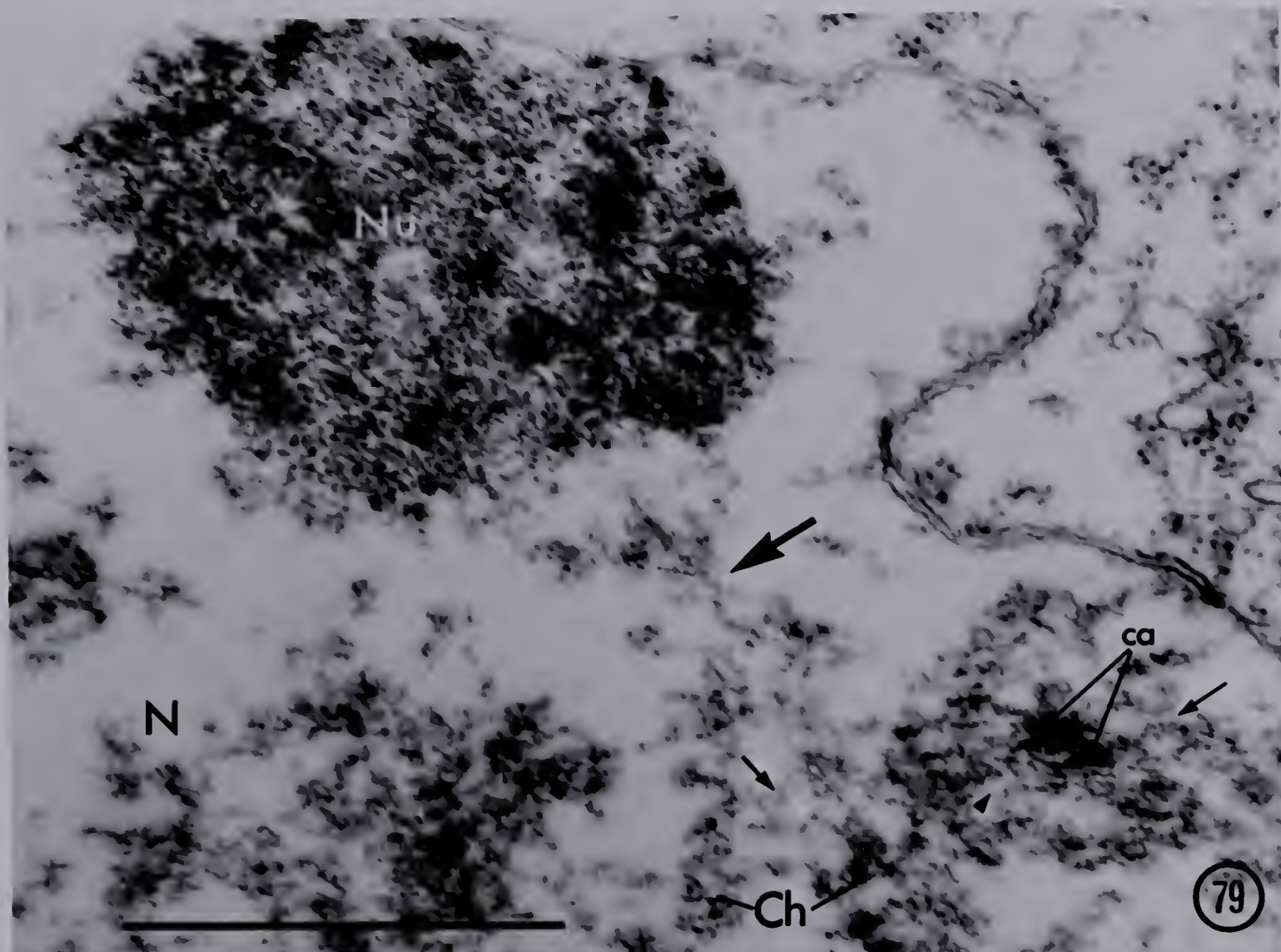
The fine structure of the lateral loops has not been investigated to any extent. As shown in Figs. 78 and 80 (arrows), they exhibit a bumpy appearance. It is suggested that the strands of the lateral loops are either coiled or folded individually.

## DISCUSSION

### Golgi Apparatus and Membrane Systems

The Golgi body of Coprinus was first recorded by
means of light microscopy by Sass (1934). This author believed
that it moved down to the bottom of the basidium and divided into
four parts. In the light of the present observation, the Golgi
body described by Sass may be quite genuine.

The presence of a Golgi dictyosome in fungi was first
demonstrated by means of electron microscopy in an ascomycete
by Moore and McAlear (1962b, 1963a). Later, similar observations
were reported for some phycomycetes (Hawker, 1963), some basid-
iomycetes (Moore, 1963c), and some myxomycetes (McManus, 1965).
Wells (1964a) described parallel double lamellae in the probasidia
of Exidia nucleata, a tremellaceous fungus. Wells expressed
doubt that lamellar systems he observed can be considered identical
to the Golgi apparatus. He suggested that parallel lamellae
"are merely a series of folds in the endoplasmic reticulum",
However, evidence is now presented that a Golgi dictyosome,
analogous to that of higher plant and animal cells, is indeed
present in fungal cells.

The Golgi apparatus as outlined by Moore (1963c) is
characterized by having: (1) stacks of sacs, (2) propinquity
to a nucleus, (3) marginal vesicles, and (4) no ribosomes

attached.  The last criterion may not be a definitive one for
fungal material.  Firstly, ribosomes of fungi are mostly free;
the endoplasmic reticulum is quite smooth, in fact.  Secondly,
in the present study, ribosomes were observed in association with
some parts of the cisternae of a Golgi complex.  This is inter-
preted as the random distribution of ribosomes in the cytoplasmic
matrix.  Thus the presence or absence of ribosomes cannot fully
distinguish parallel lamellae from Golgi cisternae.

In the basidium of Coprinus lagopus, parallel lamellae
have been observed occasionally.  This structure consists of
two cisternae (Fig. 6).  Whether or not this structural feature
is merely the folding of an endoplasmic reticulum (as the result
of chance) or of a naturally occurring arrangement remains to
be determined.  Parallel lamellae were observed in the same
section along with a Golgi complex.  This offers an opportunity
for direct comparison of the two.  From a morphological point
of view, it appears justifiable to consider these two structures
as separate entities.

The Golgi apparatus of Coprinus lagopus appears to
be continuous with the endoplasmic reticulum which, itself,
appears to be continuous with the outer nuclear membrane.  Although
in the present study the endoplasmic reticulum has not been
observed to be continuous with the plasma membrane, continuity
has been demonstrated in a discomycete by McAlear and Edwards
(1959).  These observations are in good agreement with the

concept that the Golgi cisternae and endoplasmic reticulum, together with the nuclear membrane and plasma membrane, form a complex membrane system which allows intracellular communication (Dalton, 1961). The fact that the Golgi apparatus is continuous with the endoplasmic reticulum, which is connected to the vacuoles and to the plasma membrane, may account for the certain observations.

(1) Neutral-red goes into the Golgi cisternae as it does into the vacuole. This may explain why in earlier histochemical investigations, the Golgi body and the vacuole were not distinguishable (Bose, 1931).

(2) When oil is fed to an animal, the oil moves to, and accumulates in the Golgi cisternae (see Dalton, 1961 for review).

The ontogeny of the Golgi complex is not known. Mercer (1962) suggested that a Golgi complex results from the swelling and bursting of phospholipid spherules. This hypothesis is not compatible with the Golgi complex described in the present thesis. Other workers have suggested that the Golgi complex may evolve from the nuclear membrane (Moore and McAlear, 1962b, 1963a), or from the endoplasmic reticulum (Whaley et al., 1964). Although folding of the endoplasmic reticulum has been observed in the present investigation, the possible relation of folding to the Golgi complex is not known. The ontogeny of this organelle remains obscure.

The Golgi apparatus in higher plant and animal cells has generally been thought to have a secretory function. Because there is no evidence of any secretory activity in fungal cells, at least during the development of the basidium, its function or its role in basidial development may be other than secretion.

It is assumed that the activity of the Golgi complex may be reflected in its morphological changes in relation to basidial development. In higher plants, the Golgi dictyosome is found to change its activity according to the development of the tissues (Mollenhauer, 1965; Mollenhauer et al., 1961; Whaley et al., 1964). In the present investigation, the Golgi complex was found to increase its activity tremendously at metaphase I of meiosis in the production of vesicles. Such a change of activity of the Golgi complex is noteworthy. The Golgi apparatus at this period becomes extremely proliferated as has also been reported by Moore (1963) in Puccinia, and by Manton (1960) in Anthoceros. Manton finds that the Golgi proliferation occurs only at metaphase of mitosis.

Because the production of Golgi vesicles takes place only sparsely at meiotic prophase, and profusely at metaphase, the change of Golgi activity in connection with basidial development may imply some functional significance. In the process of meiosis, there are two consecutive nuclear divisions to take place. One may suppose that the vesicles produced may be incorporated into the new nuclear membranes. This concept

is strengthened by the observation that the Golgi vesicles are closely associated with the spindle zone (Figs. 8, 58). In addition, Golgi vesicles have been seen to be incorporated into the plasma membrane (Mollenhauer et al., 1961, 1963; Porter and Machado, 1960; Drawert and Mix, 1962). It is probable that the vesicles may well be the precursors of the membrane system. This suggestion is in good agreement with the finding that vesicles are involved in cell plate formation (Porter and Machado, 1960; Whaley et al., 1963; Whaley et al., 1964). It is also compatible with the finding of Barer et al., (1960) that the vesicles join to organize the nuclear membrane at telophase. Similar and more convincing evidence was provided by Harris and Mazia (1962) who find that the vesicles join to form an envelope around individual chromosomes at anaphase. When chromosomes reach the poles at telophase, their surrounding envelopes join to form the nuclear membrane. It is tentatively suggested, as a working hypothesis, that a function of the Golgi complex in fungal cells is as a membrane-system generator. This hypothesis has been enunciated by Mercer (1962) and by Manton (1960). Mercer (1962) states, "the original phospholipid-containing vacuole (Golgi body?) may be regarded as the cell organelle for the production of and dissemination of membranes required for special purposes".

The Golgi complex of plant cells was most intensively investigated in relation to cell-wall formation (Mollenhauer et al., 1961, 1962; Drawet and Mix, 1962; Porter and Machado, 1960; Whaley et al., 1963). Specific examples that may be

noted are those in connection with the development and different-
iation of root epidermal and root cap cells.  Details of these
investigations have appeared in a recent review (Whaley, Kephart
and Mollenhauer, 1964).  These authors noted that "the most
conspicuous organelle change associated with differentiation of
the epidermis is one involving a distinct activity of the Golgi
apparatus" (p. 143).  The change lies in an increased production
of Golgi vesicles.  These vesicles are then involved in cell-wall
secretion.  In addition, these authors also noted that, during
the time when the Golgi apparatus is hypertrophied, the endoplasmic
reticulum is abundant.  In contrast, the endoplasmic reticulum
is relatively sparse in root-cap generative cells before hyper-
trophy occurs.

These observations appear to point to two possible
functional activities of the Golgi complex; (1) a secretory
function, and (2) membrane production.  Whaley et al., (1964)
are reluctant to accept the second possibility.

No doubt the Golgi complex may be involved in sec-
retion in some cell types, but this is not necessarily true
for all cell types.  As Dalton (1961) has pointed out in an
extensive review, secretion appears to be associated with
some cell types but definitely not with others.

The present hypothesis is not necessarily incompatible
with the suggestion that Golgi vesicles are involved in the
secretion of zymogen in animal cells (Palade, 1959; Ichikawa,

1965), and the secretion of cell wall deposition granules in plant cells (Mollenhauer et al., 1961; Whaley et al., 1964; Drawer and Mix, 1962; Cronshaw and Bouck, 1965). The vesicles which contain the zymogen granules or the wall deposition granules were observed to join with the plasma membrane before they discharge their contents outside the cell membrane.

It is established that the endoplasmic reticulum is involved in the synthesis of enzymes (Palade, 1959). It may very well be that the Golgi vesicles produced by the Golgi complex are only precursors of the membrane system. The membranes then synthesize substances (such as zymogen granules) which accumulated inside the cisternae. This contention is in agreement with the evidence presented by Palade (1959), and more recently by Ichikawa (1965), that the contents of the vesicles increases gradually after the latter are released from the Golgi zone. Further evidence was presented by Cronshaw and Bouck (1965) and Palade (1959) that there are two types of vesicles; one has electron-absorbing contents, and the other has not. It is interesting to note that the vesicles with contents are not so closely associated with the dictyosomes as are those without contents (Cronshaw and Bouck, 1965). This is taken to mean that the Golgi complex is not the primary site of the synthesis of zymogen granules. This is a reasonable assumption from the morophological point of view. This view is in agreement with the hypothesis of Novikoff et al. (1962) that lysosomes arise from Golgi vesicles, and it is further strengthened by the finding of Moe et al. (1965)

that the "virgin lysosomes" are the products of the Golgi com-
lex.   In addition, this assumption is compatible with the fact
that in subcellular fractionation, the Golgi fraction exhibits
very few enzyme activities (Dalton, 1961).   Acid phosphatase
activity is associated only with one of the Golgi cisternae
(saccules) which is usually one of the outer cisternae (Moe et
al., 1965).

It may be contended that vesicles without contents
are produced from the ends of Golgi cisternae.   The Golgi ves-
icles, when released or about to be released from the Golgi region,
are able to undertake the function of synthesis.

First, in case of exocrine cells or of pancreatic
cells, zymogen granules are produced by and stored in the ves-
icles.   These vesicles then join with one another and with the
plasma membrane to form a canaliculus through which the contents
may be discharged (Palade, 1959; Ichikawa, 1965).   Subsequently,
the membrane becomes integrated as part of the whole membrane
system.

Second, in the case of the basidium of Coprinus, where
no secretion is evident, and where three nuclear divisions (meiosis
and mitosis) and spore production are to take place; the Golgi
vesicles accumulate in the cytoplasm and no contents are evident
in these vesicles.   The vesicles may be directly incorporated into
the nuclear membrane at telophase or they may anastomose to form

the endoplasmic reticulum.

The plasma membrane has been suggested to be the source of all endomembranes (see Giese, 1962 for review). Although invagination of the plasma membrane has been observed in the present investigation, such invagination is not sufficient to account for the increase of cytoplasmic vesicles as well as endoplasmic reticulum in developing cell, as was pointed out by Giese (1962). In contrast, the proliferation of the Golgi complex at metaphase I provides good evidence that the Golgi complex may be regarded, more satisfactorily, as the source of these membranes.

However, one cannot preclude either one possibility or the other. In fact, vesicles have been seen to arise from the system of parallel lamellae, or from the folded endoplasmic reticulum. It would seem that vesicles may arise from all membranes, by invagination of the plasma membrane (see Giese, 1962), or by blebbing of the nuclear membrane (Moore and McAlear, 1962a, 1963a; Wischnizer, 1963). Vesicles arise from the sources mentioned above are not as obvious as from the Golgi complex, however.

## Lomasomes

Lomasomes were first named by Moore and McAlear (1961a) who suggested that these organelles might be concerned with the metabolism of the cell wall. Girbardt (1958) suggested that the

"rings" (lomasomes) might represent the plasma pore; he was
not fully satisfied with this explanation however.

In the present investigation, lomasomes were found
only rarely in a young basidium. They are, however, rather
abundant in older basidia in which meiosis has been completed.
This observation is in agreement with what Girbardt (1958)
found in Polystictus versicolor, where larger "rings" were
associated with the older mycelium. These observations may be
taken to mean that Moore and McAlear's suggestion is tenable.
Moreover, the observation that lomasomes within the basidium
bear a close resemblance morphologically to the multivesicular
organelle (the lysosome) may suggest that the lomasomes may be
lysosome-like organelles which consist of hydrolytic enzymes
responsible for cell wall breakdown.

## Mitochondria

It is well established that the mitochondria of fungi
resemble those of higher organisms (Moore and McAlear, 1963b;
Wells, 1964a; Girbardt, 1958; Luck, 1965a; Shatkin and Tatum,
1959). These organelles are extremely abundant in the basidium
of Coprinus as can be verified both by phase contract microscopy
and by electron microscopy. Under direct observation with the
phase-contrast microscope, mitochondria were observed to be very
active around the nucleus during nuclear division. This observa-
tion is now confirmed by electron microscopy (Fig. 51).

It is interesting to note that the distribution of
these organelles in the basidium displays a clear polarity --
there is an abundance of them in the upper half of the cell but
there are only a few in the lower half.  Mitochondria are living
power houses, and it would seem that they are gathered to where
a supply is most needed.  In the upper half of the basidium a
nucleus is to undergo meiosis, a Golgi complex is to produce
vesicles which may be needed for the nuclear membrane to be
re-built, and much protein synthesis is being carried on to meet
the needs of basidiospore production.

The mitochondria in the basidium of  Coprinus vary
greatly in size and shape.  Similar variation has been found in
animal mitochondria (Novikoff, 1961).  This variation may be
explained on the basis of the plasticity of the mitochondrial
membrane.  In a recent report, Stephens and Bils (1965) described
an atypical form of mitochondrion.  This atypical form may be
the result of the invagination of the mitochondrial membrane
which, in turn, is an outcome of membrane plasticity.

## Fruit Body Development

From the evidence presented by electron microscopy,
it is apparent that the development of a fungus fruit body has
a unique pattern of subcellular organization and differentiation.
With some extrapolation, one can envisage the sequence of events
as follows.  A fruit-body primordium is formed from a mass of
mycelium which probably arises from a single dikaryotic cell.

In the fruit-body primordium all mycelial branches are equally
actively engaged in cellular activity and growth; and at this
stage no differentiation at the subcellular level is discernible.

As the fruit-body primordium increases in size, cellular
organization and differentiation begin.  Some hyphae become organ-
ized into the stalk, others form the gills or the hymenium.
Among the hyphae of the hymenium, some become the generative
cells from which basidia are formed, others form cystidia and
the cortical pseudoparenchyma.

The basidia, being the site of spore production, are
of central importance in fruit-body development.  It is not sur-
prising that the electron microscope reveals more subcellular
particles in the basidia than in any other cells connected with
them.  If the concentration of subcellular particles is an index
of the metabolic activity of any cell, then the cells remotely
connected with the basidia are very much less active metabolically
then are those closely associated with the basidia.  The inactive
cells of the mycelium are depleted of subcellular particles, and
the food supply is reduced to the minimum.  Food materials and
subcellular particles are channelled into the basidia, or into
cells closely associated with them.

As the basidia develop, the active cells gradually
become inactive until, eventually, all cells except the basidio-
spores have become inactive.  The fruit body then undergoes
autodigestion.

The development and differentiation of the cystidia of _Coprinus_ is of interest. Microscopically, these giant sterile cells serve as pillars to ensure the functional architecture of a gilled fruit body. They probably play an important role in facilitating the dispersal of spores from basidia (Buller, 1931). Submicroscopically, these multinucleate giant cells have a unique subcellular organization during development. At an early stage of development of a fruit body, cystidia have few ribosomes, but many mitochondria. This is taken to mean that cystidia are not active in protein synthesis, but are active in oxidative metabolism. In the course of development, cystidia become extremely vacuolate and the number of mitochondria in these cells decreases, suggesting a decrease in metabolic activity. At the final stage of development (spore-producing stage) there is an abrupt change in the subcellular organization of the sterile cells, marked by the presence of osmiophilic granules in the cytoplasm of these giant cells; the granules may be lipid globules.

Similarly, the accumulation of lipid globules in basidiospores is further evidence of metabolic regulation. Throughout the course of fruit-body development, lipid globules are very scarce in the basidium, whereas (at the spore producing stage) there is abrupt increase to as many as ten times in the number of lipid globules over a short period of time. This indicates that the lipid-condensing enzymes are metabolically

regulated for release precisely at the moment appropriate for the accomplishment of this important morphogenetic shift.

The discussion above is based solely on the evidence provided by subcellular organization as shown by the electron microscope; more direct proof regarding metabolic regulation could be obtained from enzymic and biochemical studies. However, the cytological evidence presented in the present thesis may be useful for suggesting subsequent physiological investigation.

## Meiosis

The behavior of nuclei in a developing basidium has been described for the higher basidiomycetes (Lu and Brodie, 1964; Lu, 1964a). Chromosomes of the two compatible nuclei examined with light microscopy were interpreted, on the basis of their long and thread-like nature, as being in late telophase or interphase. The interpretation is consistent with the present results obtained by electron microscopy which indicate these pre-fusion nuclei also closely resemble the telophase-interphase nuclei in the even distribution of the chromatin fibrils within the nuclear membrane.

Although the fusion of two compatible nuclei has been observed with the light microscope (Lu, 1964a), their nuclear membranes were not visible. From a study of the electron micro-graphs presented here, it appears probable that fusion is accomplished by dissolution and reassembly of the nuclear membranes at the point of contact of the two haploid nuclei.

After nuclear fusion, synapsis would be expected to follow. This assumption is borne out by the observation that the synaptinemal complex is present before the fusion of two nucleoli (Fig. 50). This interpretation is compatible with the established fact that the complex is present only at the synaptic stages, and it comprises the intimately paired bivalent (Moses, 1958; Ris, 1961; Meyer, 1960; Moses and Coleman, 1964). This interpretation that the synaptinemal complex of Coprinus may also represent a bivalent is strengthened by direct comparison between bivalents as revealed by light microscopy (Fig. 39) and the synaptinemal complex (Figs. 53, 54, 74).

The details of the mechanism of synapsis are not thoroughly understood and probably cannot completely be resolved by techniques dealing with fixed materials regardless of whether the light microscope or the electron microscope is used. Nevertheless, information obtained from comparative studies using the light and the electron microscope may provide some clues regarding the process. First, since the synaptinemal complex occurs only at the synaptic stage, one may assume that the process which brings about the patterned structure of this complex is the process involved in chromosome synapsis. This assumption is based on the fact that the chromatin fibrils, which are loosely and evenly distributed in interphase nuclei (Fig. 49), have become organized into the elaborate structure of the synaptinemal complex.

Secondly, since the synaptinemal complex consists of two homologous chromosomes, each of which results from the folding of two chromatin fibrils, the process of folding of the chromatin fibrils to form a synaptinemal complex may be equated to the process of chromosome synapsis. As synapsis proceeds, a chromosome shortens to a specific length, typical of that chromosome at the pachytene stage. This fits well with the observation that a fusion nucleus has long tangled chromosomes before synapsis is complete (Figs. 35, 36), and that chromosomes are relatively short (Fig. 37) when synapsis is complete.

In an earlier report (Lu, 1964a) it was suggested that "as synapsis proceeds, chromosomes elongate to facilitate point by point pairing". This concept is incompatible with what may be inferred from the fine structure of the synaptinemal complex, and appears now not to be correct. As revealed herein by light microscopy, the diploid nucleus consists of one nucleolus, and a mass of extremely long, thread-like chromosomes. Whether or not these chromosomes are paired cannot be determined by light microscopy. The fact that, in resolvable regions, they are of the same thickness as those in the pre-fusion nuclei suggests that synapsis is not yet complete. It seems probable that they do not elongate during the process of synapsis. However, this does not exclude the possibility that chromosomes may elongate before the formation of the synaptinemal complex.

At pachytene and diplotene, chromosomes become condensed. Bivalents can be demonstrated both by the light

the surface of these ovoid structures may be considered simi-
lar to those of the centriole of <u>Albugo</u> <u>candida</u>  as reported
by Berlin and Bowen (1964).  It is probable, therefore, that
they are indeed centrioles.

        Their intimate association with the outer nuclear
membrane is of particular significance in the fungal system.
Such relationship would appear indispensable for retention
of the centriole during the nuclear migration that occurs in
basidiomycetes (Snyder and Raper, 1958; Giesy and Day, 1965)
and for nuclear streaming (as in <u>Neurospora</u>, Lu, unpublished).
The triple overlapping of the nuclear membrane suggests that
the outer membrane of the three overlaps the centriole, as is
shown in the electron micrograph of Moore and McAlear (1962c).

        The observation that spindle fibers may be formed
between a pair of centrioles, without the association of meta-
phase chromosomes, indicates the existence of a structural
spindle.  Thus before the metaphase orientation of chromosomes,
this is formed between two centrioles then allows chromo-
somes to be attached to the spindle during the gathering of
chromosomes at the equatorial plate.  This interpretation is
strengthened by Girbardt's study of <u>Polystictus</u> <u>versicolor</u>
(1962) in which it was demonstrated that a "bar" was attached
to the chromatin mass.  Girbardt's bar may be the structural
spindle suggested in the present thesis.  Its existence is
supported  by the observation that, at late anaphase when all

chromosomes have reached the poles, a spindle remains visible (as can be seen in Fig. 45).

## The Organization of Nuclei

Although the presence of nuclear annuli has been established in fungi recently (Wells, 1964a; Moore and McAlear, 1962a; Girbardt, 1958), their fine structure has not been understood. The present observation of microtubules around the annuli similar to what has been described for animal cells, suggests that nuclear annuli of fungi are homologous with those of higher organisms. The information concerning nuclear annuli of animal cells may be found in the literature (Bernhard, 1959; Mirsky and Osawa, 1961; Kessel, 1965; Afzelius, 1955).

Regarding the pore proper, there is a controversy as to whether or not there exists a diaphragm which controls nuclear-cytoplasmic communication (Bernhard, 1959; Mirsky and Osawa, 1961). In the present thesis, the probable 9-2 fibrillar arrangement may suggest that the nuclear pore is not just a hole; rather it has a definite structure, at least insofar as Coprinus lagopus is concerned. This is in good agreement with the recent discovery that only particles with diameters of 145 Å or less can pass through nuclear pores (Feldherr, 1965).

Regarding the chromatin fibrils, there is controversy as to the extent or degree to which they are composed of strands. It has not been possible, in this study, to re-

solve the argument between some workers who contend that a

200 $\overset{o}{A}$ fibril consists of two 100-$\overset{o}{A}$ fibrils each of which, again,

has two 40-$\overset{o}{A}$ subunits; and others who contend that the fibril

is single, because of the limited resolution. In some favor-

able areas, a 100-$\overset{o}{A}$ fibril has been observed to result from

the coiling of a single 20-30 $\overset{o}{A}$ fibril. Observation from

the limited favorable areas available does not constitute

sufficient evidence to decide between the alternatives.

However, observation of interphase nuclei of cystidia

merits special attention. These special somatic nuclei differ

from others in that their chromosome continuity may be ob-

served. As is shown, a chromosome consists of foldings of

chromatin fibrils closely comparable to the folded-fiber model

of DuPraw (1965).

The chromosome which is attached to the nuclear

membrane (Fig. 73) appears to consist of two 100-$\overset{o}{A}$ fibrils.

Such fibrils are suggested to consist of only one 40-$\overset{o}{A}$ fibril

each, in agreement with what has been shown in honey-bee by

DuPraw (1964, 1965). As is known, this particular nucleus

does not divide again before autolysis of the fruiting body

occurs. Whether this nucleus represents a pre-replication

or a post-replication interphase is not certain. If it is

post-replication, then a chromosome consists of two strands

of DNA protein fibrils, each being a chromatid; that is to say

there is no half-chromatid in the somatic nucleus of Coprinus.

On the other hand, if it is pre-replication, then a chromatid would consist of two DNA-protein fibrils after DNA replication, each representing a half chromatid.

It should be pointed out that the evidence concerning the one versus the two fibrils presented in the present thesis is circumstantial only. It should be taken as suggestive rather than as conclusive evidence. Further investigation is necessary to establish this point.

## The Organization of Meiotic Chromosomes

Since the synaptinemal complex has not been observed before and after the synaptic stages (zygotene-pachytene), and since the metaphase chromosomes do not exhibit any axial differentiation (Nebel and Coulon, 1962a), the question has been raised as to what the transitional changes from a synaptinemal complex to a condensed metaphase chromosome may be. The demonstration of the diplotene chromosome (Figs. 57, 79), provides an interesting clue to the above problem. It is apparent that the structural materials of the homologous-chromosome axes and those of the lateral loops are the same, viz. the microfibrils (Figs. 74, 76). It is suggested that the difference in electron density is primarily due to the degree of aggregation of microfibrils resulting from the pattern of foldings of the chromonemata. The concept of foldings of fibrils to form a chromosome is not incompatible with the model proposed for a lampbrush chromosome

(Lafontaine and Ris, 1958; Swift, 1962). It is thus possible
that the diplotene chromosomes of Coprinus may be a miniature
lampbrush type.

In the transverse section of a synaptinemal complex,
the homologous-chromosome axes appear as two distinct cylinders
(Figs. 75, 77, 79). This view gains support from the electron
micrographic studies of Moses and Coleman (1964) and Nebel and
Coulon (1962a). Further, Nebel and Coulon (1962a) demonstrated
that the axes of two sister chromatids of a homologue are sep-
arated at a later stage. However, Sotelo and Wettstein (1964)
contended that the cross section shows the minimum width of the
three components. This view is not consistent with the present
finding. As a matter of fact, the short segments of three parallel
elements have been observed. They represent a longitudinal
section of a very short segment of the synaptinemal complex,
however.

There is a definite pattern in the organization of
chromatin fibrils into a synaptinemal complex. In the longitud-
inal section as illustrated in Fig. 74 and 76 and especially
clearly in Fig. 54, the microfibrils are arranged preponderantly
at right angles to the chromosome axis. In the transverse section,
moreover, the microfibrils appear to have a common center from
which they radiate (Figs. 76, 77). The observations agree with
what has been demonstrated by Moses and Coleman (1964) and by
Nebel and Coulon (1962a), but disagree with the report of Sotelo

and Wettstein (1964) who stated, "no specific pattern of distri-
bution is noticed in the filaments (fibrils)* composing the lat-
eral arms (homologous-chromosome axes) of the group (synaptinemal
complex) shown in any of these (longitudinal and transverse)
views".  The results of the latter authors are difficult to
assess.

It has been established that the meiotic process in the
basidiomycetes as revealed by light microscopy, resembles that
of higher organisms.  The demonstration of a synaptinemal complex
in a fungus with all the characteristics of this structure in
higher organisms, suggests strongly that the fungi are more sim-
ilar in meiosis to those organisms than has hitherto been thought.
The suggestion from the work of Meyer .(1960,1964 as cited by Moses
and Coleman, 1964) that the synaptinemal complex is more specif-
ically involved in synapsis for crossing-over than in pairing
for disjunction (distributive pairing, Grell, 1962, 1964) may
be extendable to higher fungi.  Thus it is suggested that the
fungus Coprinus and the higher organisms share a common mechanism
for crossing-over.

The Organization of a Synaptic Chromosome -- A Model

To date, several models have been proposed to explain
the organization of a meiotic chromosome.  The earlier ones
were based on observations of the lampbrush chromosomes of

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* text in brackets inserted by the present author to explain
  the terminology.

amphibians. No doubt, this giant chromosome has a unique role in the investigation of the chromosome organization.

As pointed out earlier, the protein-backbone theory has not gained general acceptance. In fact, one of the original supporters has now abandoned the concept (Taylor, 1964). However, on the basis of their observations of the tripartite synaptinemal complex and on the evidence that the complex proper is DNase-resistant (Nebel and Coulon, 1962b), Nebel and Coulon (1962a) recently proposed a model in support of the protein-backbone theory. In this model the lateral loops are inserted on the lateral component (the homologous-chromosome axis) which is con-sidered as the backbone. This model does not accommodate genic linearity unless it is postulated that each lateral loop is a cistron, which is highly unlikely. If the assumption of Meyer (1960, 1964) that the synaptinemal complex is directly involved in crossing-over is acceptable, then crossing-over would occur only at the pairing surface where two homologues meet. According to the chromosome structure visualized in the present study, crossing-over could occur only in paired parts of the lateral loops. In this regard, Nebel and Coulon's model provides dif-ficulties with respect to crossing-over and chiasma formation and to chromosome or chromatid exchange. In addition, this model is incompatible with the findings of DuPraw (1964, 1965) who demonstrated that an interphase chromosome consists of long single fibers.

The multistrand or rope model is the one most often advocated, as may be noted in several reviews (Ris, 1961, 1962; Steffensen, 1959; Kaufmann et al., 1960). This concept has been reiterated recently by Sparvoli, Gay and Kaufmann (1965). As Schwartz (1960) has pointed out, "a multistranded chromosome imposes many difficulties in regular chromosome duplication and separation after crossing-over, as well as breakage and reunion". In view of the fact that there is little evidence from electron microscope studies of multistrandedness. The multistranded model is still of doubtful validity.

In the present thesis, I have presented the fine structure of a bivalent as revealed: (1) by frontal sections, (2) by sagittal sections, and (3) by cross sections. The question is how can one compound these three kinds of sections into a view of a three-dimensional chromosome. In thin sections, the continuity of the structural element, the microfibrils, is severed; only in a favorable area may the continuity be observed or suggested. Such favorable areas do not always constitute satisfactory evidence.

Whole mount electron microscopy has its merit. It does not sever the continuity of the microfibrils, and therefore, it may provide information lacking in thin sections. Whole-mount electron microscopy has not been applied to fungal chromosomes. Information as to how whole fungal chromsomes, especially the pachytene chromosomes, would appear as revealed by the

electron microscope is not yet available.  However, one may
gain some clues from the observations on honey-bee chromosomes,
if it is assumed that chromosomes of different species have some
common features.

     In the following, I shall attempt to describe, with
the aid of schematic diagrams, a model of the possible organization
of a three-dimensional pachytene chromosome.  This model is based
on careful observations of the synaptinemal complex as revealed
by thin-section electron microscopy.  It is also based on the
following assumptions (1) that a single deoxyribonucleoprotein
fibril runs throughout the length of a chromosome (for a moment,
the strandedness of this fibril is left an open question); (2)
that the foldings of this fibril depend on some kind of molecular
bonding which probably resides within the protein associated
with the DNA molecule.

     As suggested earlier, the DNA-protein fibril folds to
form a pachytene chromosome in such a fashion that each fold
passes through the vicinity of the axial area.  Such a folding
is illustrated in Fig. 81 which is drawn to simulate a transverse
section of a synaptinemal complex.  It can be seen that this re-
presents a single chromatid of the homologues.  It is believed
that two chromatids of a homologue  fold together but do not
coil around one another at pachytene.  The kind of folding
described above permits the folding center of the chromatids
to move apart at a later stage as shown by Nebel and Coulon (1962a).

Fig. 81.   A diagrammatic drawing of a view of the cross
           section of an idealized synaptinemal complex
           (or the synaptic bivalent) which is made by
           folding an artificial fiber in a manner that
           each fold passes through the vicinity of the
           axis.  The two axes represent the homologous
           chromosome axes, from which lateral loops
           radiate.  The overlapping of short loops
           between two axes represents the synaptic
           center.


Fig. 82.   A diagrammatic drawing of a view of the longi-
           tudinal section of an idealized synaptinemal
           complex.  The section is cut through the dotted
           line indicated in Fig. 81.
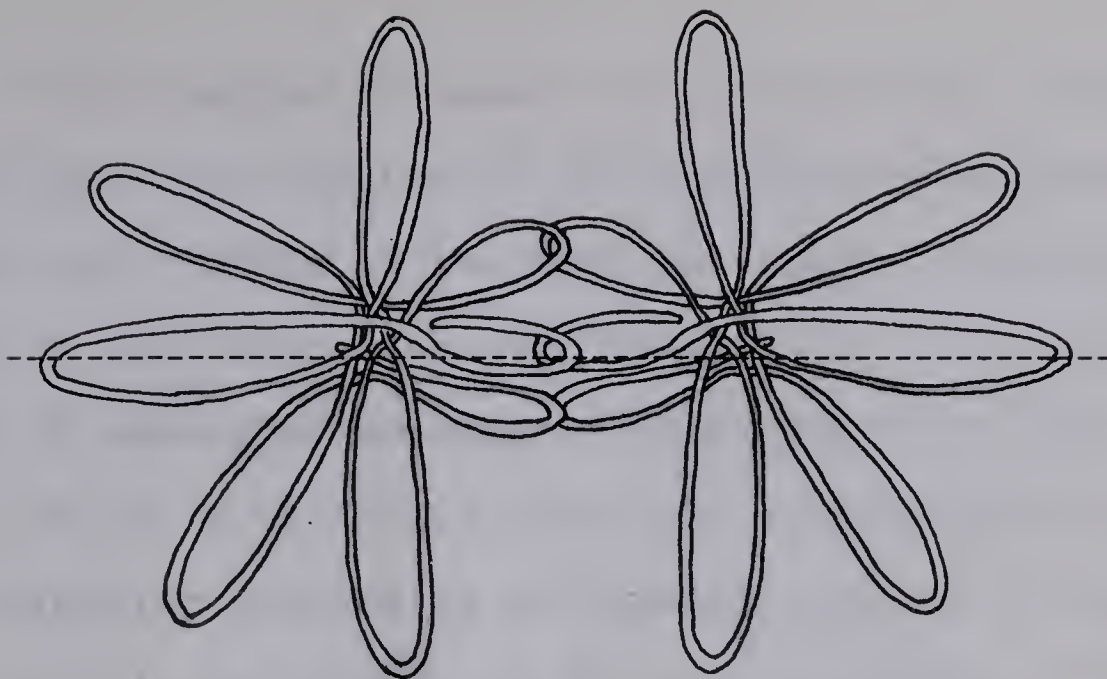
Fig. 81
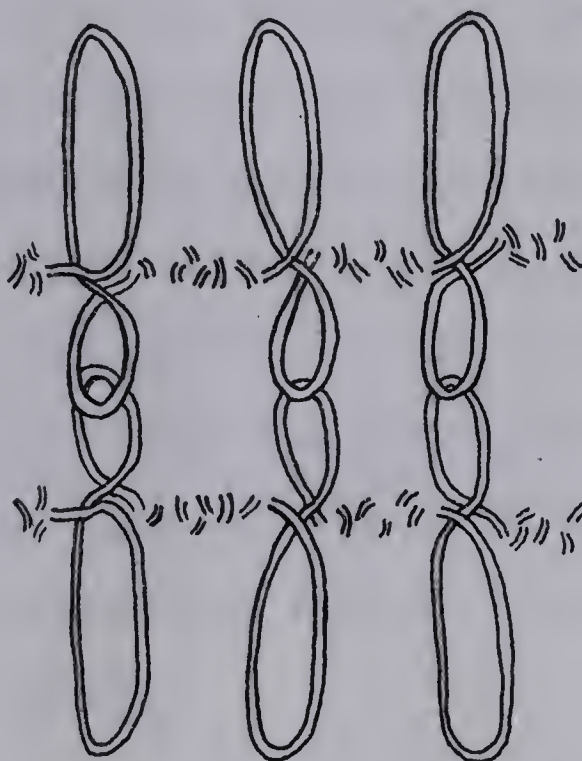


Fig. 82

This diagram stresses the concept that the homologous-chromosome axes are the result of fibrillar aggregation. This means that the fibrils of the axes and those of the lateral loops are parts of the same continuous fibril. It is postulated that some kind of molecular bonding is responsible for the pattern of folding. It is in the axial area that such bonding occurs. The kind of molecular bonding is not predicted; it is suggested, however, that it may reside in the protein moiety of the DNA-protein complex.

The concept that the foldings of the chromatin fibril have a molecular basis stems from the following: (1) genetically, the foldings of the homologues demand an identical and specific pattern in order to explain specific genic pairing at the synaptinemal complex; and (2) cytologically, each chromosome has unique morphology. For example, among ten chromosomes of Coprinus lagopus, the two largest ones have an acrocentric constriction (Figs. 40, 41). It is suggested that in this area bonding components are probably lacking.

Fig. 82 illustrates an idealized 200 Å thick longitudinal section of the structure represented in Fig. 81 along the dotted line. It can be seen that the lateral loops appear loose. In between, are shown short fibrils which are arranged at right angles (or near so) to the long axis; these represent lateral loops, the tips of which have been cut off in the preparation.
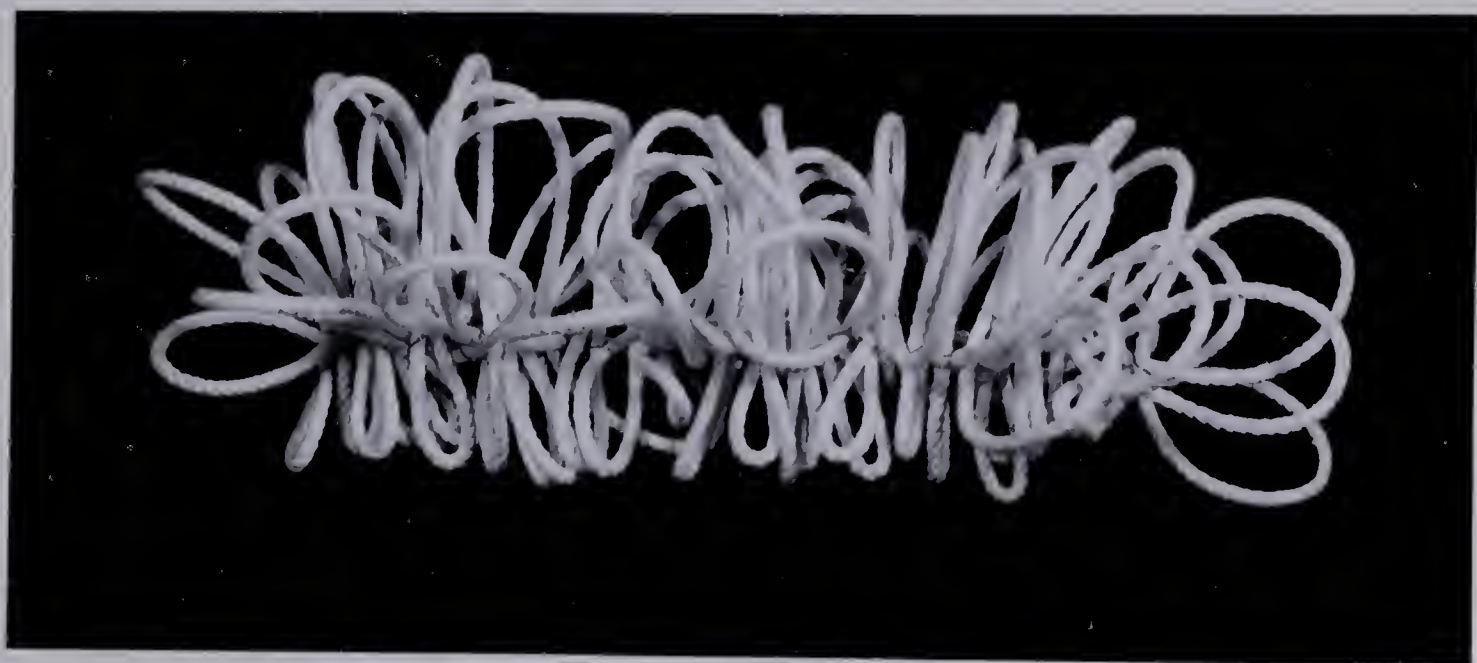
Fig. 83. A photograph of the proposed model of a
chromosome which is made by folding an artificial
fiber in such a fashion that each fold passes
through the vicinity of the axis of the structure.
When two of these are stuck together, they give an
appearance of a synaptinemal complex.

Regarding the synaptic center (pairing surface), this model, as can be seen in Fig. 81 and Fig. 82, suggests localized pairing which is in agreement with the concept of "effective pairing' of Pritchard (1960, this concept of effective pairing is quoted and accepted by Moses and Coleman, 1964). Although the synaptic center consists of loop-ends as shown in Fig. 74, actual pairing of two homologous loops has not been observed. More evidence is necessary.

A three-dimensional reconstruction of the model, made by folding an artificial fiber according to the foregoing description, is shown in Fig. 83. This photograph bears a good resemblance to the chromosome of honey-bees as revealed by whole-mount electron microscopy (DuPraw, 1965).

This model is closely related to the folded-fiber of DuPraw (1965), the applicability of which with respect to duplication, genetic and cytogenetic behavior has been discussed by DuPraw (1965). Although the present model is compatible with most cytological and genetic data, it does not seem to explain interference. Whether or not orderly foldings may account for chromatid interference remains to be explored.

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